Four wings, two wings, no wings – patterns of wing reduction in Holometabola (Insecta)

Dissertation

To Fulfill the
Requirements for the Degree of
„doctor rerum naturalium“ (Dr. rer. nat.)

Submitted to the Council of the Faculty
of Biological Sciences
of the Friedrich Schiller University Jena

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born on 13.02.1988 in Beijing

Dissertation, Friedrich-Schiller-Universität Jena, [2018]

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If you're sad, add more lipstick and attack.

-- Coco Chanel

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1. Introduction

The acquisition of the wings made Pterygota extremely successful in accessing three-dimensional space, especially plant surfaces. This made it easy to reach scattered food resources and also provided a critical escape mechanism when under threat (Wagner & Liebherr 1992; Beutel et al. 2017a). The insects of this monophylum rapidly diversified to become a dominant group on land from mid- to late Carboniferous and retained this status to the present time (Dudley 2001). There are two prominent interpretations concerning the wing origin (Chlebak 2013): The static tergal projection or paranotal lobe theory is based on some insect fossils from the Carboniferous, which displayed small plate-like outgrowths of the pronotum in addition to pterothoracic wings (Šulc 1927; Snodgrass 1935; Hennig 1969; Manton 1977). The alternative hypothesis suggests that proximal leg appendages were gradually transformed into functional wings (Kukalová-Peck 1983; 1987). Recently investigations in developmental biology remained ambivalent, providing tentative support for both alternatives (Linz & Tomoyasu 2018).

With the evolution of Pterygota in the late Palaeozoic and early Mesozoic, various flight modes appeared in different groups, such as functionally four-winged flight or different varieties of two-winged flight (Brodsky 1994), anteroposteriorly symmetric or asymmetric flight (Wootton 2001). However, a general mode of flight can still be reconstructed in detail based on the homology of the thoracic structures related to wing function and specifically the flight apparatus itself. The complete wing stroke cycle can generally be divided into four distinct stages: depression, supination, elevation and pronation (Brodsky 1994). These stages are strictly controlled by various skeletal and muscular elements combined in the pterothorax.

Insect flight structures function in a similar pattern in almost groups, with the noteworthy exception of Odonata (Kozlov 1986c; Brodsky 1994): The large bundles of indirect flight muscles in the central space of the pterothorax mainly activate the wings. The upward movement of the wings is initiated by the contraction of dorsoventral muscles, while the downward wing stroke is caused by the contraction of the dorsal longitudinal muscles. These indirect wing muscles usually connect with the notum, pleuron, sternite or coxal rim. They function as wing levators and depressors, without being directly attached to the wing base. Sutures and sulci of the notum evolved with the flight apparatus, enabling specific patterns of deformation of during flight. Direct wing muscles are attached to the wing base sclerites. They function as wing pronator, promotor, supinator or flexor, strictly controlling wing movement via basal articulatory configurations, executing transitional movements related to the wing stroke. They are also involved in unfolding and folding the wings at the beginning and end of flight. Some direct muscles can have multiple functions, as in the case of wing levators or

depressors. Apart from their function, some flight-related muscles are also engaged in stabilizing the thoracic box. They increase the resiliency of the skeleton, and can also play a role in leg movements and walking locomotion on the ground.

The PhD project was carried out in the framework of the BIG4 program, BIG4 referring to the four extremely species rich orders of Holometabola, including Hymenoptera (> 125 000 spp.), Coleoptera (> 350 000 spp.), Lepidoptera (≈ 150 000 spp.) and Diptera (> 150 000 spp.) (Friedrich 2009). Therefore, these four groups will be briefly characterized in the following, specifically referring to thoracic features.

Holometabola: The flight apparatus groundplan of holometabolan adults is close to that of adult neopteran insects as pointed by Peters et al. (2014): The assessment of the ancestral condition of the flight apparatus (groundplan of Holometabola) is ambiguous. It is possible that approximately equally sized pterothoracic segments without wing coupling mechanism are the ancestral state, as this morphological pattern is also present in some orders of Polyneoptera (Brodsky 1994). Posteromotorism with an increased size of the metathorax is usually considered as apomorphy of the common ancestor of Strepsiptera and Coleoptera (Koeth et al. 2012), but does not occur in the polyneopteran Orthoptera and related groups. Another possible option is anteromotorism with a functionally and structurally enhanced mesothorax as the ancestral status. This is present in several orders of Holometabola including the basal branch Hymenoptera, and also in the paraneopteran Psocodea, which are possible close relatives of Holometabola (Misof et al. 2014).

Hymenoptera: A wing coupling mechanism with hamuli apparently evolved as an autapomorphy of Hymenoptera. This feature is linked with a flight mode combining anteromotorism and functional dipterism (e.g. Beutel et al. 2014; Peters et al. 2014). The mesothorax is enlarged with strongly developed flight muscles, whereas the pro- and metathorax are reduced in size, the latter fused with abdominal segement I (Beutel et al. 2014). Xyelidae, the sister group of the remaining Hymenoptera, retain the highest number of tergo-pleural muscles within the order, which is probably a groudplan feature (Vihelmsen 2000a; b; 2001; Friedrich & Beutel 2010b). Three families of Aculeata have evolved eusociality with different castes, with a functional flight apparatus retained in all morphs in Apidae and Vespidae (Snodgrass 1910b; 1942; Duncan 1939). The profound different between highly modified thoracic structures of the flightless ant workers and the preserved flight apparatus in sexual morphs of Formicidae is an obvious autapomorphy of the family.

Coleoptera: Species of Coleoptera have a metathorax much larger than the mesothorax. The forewings are transformed into sclerotized elytra for protecting the dorsal abdomen, hind wings and pterothoracic pleural regions (Dudley 2001; Beutel et al. 2014). The

pterothoracic muscular system is simplified compared with a generalized pattern of Neoptera, probably linked with the strong sclerotization and reduced degrees of freedom, especially at the leg bases (Beutel & Haas 2000; Friedrich et al. 2009). The morphological structure of Archostemata, a tiny relict group (ca. 40 spp.). is likely close to the groundplan of Coleoptera, with a lesser degree of fusion of thoracic sclerites, more degrees of freedom in the exoskeleton and a comparatively complex musculature (Friedrich et al. 2009). Simplifications of the thoracic skeleton-muscular system likely took place independently in the megadiverse Polyphaga, arguably the sistergroup of the remaining Coleoptera, in the miniaturized Myxophaga and to a lesser degree in Adephaga (Beutel et al. 2018).

Lepidoptera: The moderate flight ability in the groundplan of Lepidoptera is reflected by non-glossatan families, especially Micropterigidae, likely the sistergroup of the remaining order (Kristensen 1998). Its pterothoracic segments are almost equally sized, resulting in a moderately efficient flight apparatus (Kristensen & Skalski 1998; Kristensen 1998; Imada et al. 2011). A similar pterothoracic configuration is present in Neuropterida and Mecoptera, and is considered as plesiomorphic for Aparaglossata (Holometabola excl. Hymenoptera) (Peters et al. 2014). A successive enhancement of the flight ability is a major trend in the evolution of Lepidoptera. The flight mode is changed to anteromotorism with an enlarged mesothorax and both pterothoracic segments forming a compact unit. This increases the efficiency of flight, with high speed or long-distance migration in some taxa (Beutel et al. 2014).

Diptera: In Diptera the mesothorax is enlarged like in Hymenoptera, whereas the prothorax is small and the metathorax distinctly reduced (Beutel et al. 2014; Fabian et al. 2016). As another group with anteromoterism, in this case combined with anatomical dipterism, the flight movements mainly depend on the functional forewings, whereas the hindwings are transformed into short halters (Matsuda 1970; Beutel et al. 2014; Peters et al. 2014; Fabian 2016). The metathoracic halters are not merely largely reduced hind wings: they function as the mechanosensory control organs and stabilize flight with their rotatory movement (Chapman 1982; Fayyazuddin & Dickinson 1999; Deora et al. 2015). A complete hypothetical 3D model of dipteran flight mechanism was proposed by Pringle (1957) and Pfau (2008) emulated it with a mechanical model.

In nearly all winged insect orders secondarily flightless species have lost the immense advantage of flight (Wagner & Liebherr 1992). Previous researchers have summarized some general characters of flightless insects (Roff 1986; 1990; Wagner & Liebherr 1992). They usually live in relatively stable habitats, frequently in woodlands and deserts, few in aquatic environments or unusually cold areas, and some on animal surfaces as ectoparasites. Flightless insects are often sexually dimorphic, and loss of the flight function occurs more frequently in females, enabling a greater allocation of resources to

egg production. Summarizing accounts of morphological transformations of several flightless members of Polyneoptera and Holometabola were given by Wipfer et al. (2015) and Friedrich & Beutel (2010), respectively, including the fusion or lacking subdivision of pterothoracic tergites, reduction of wing base sclerites and flight related muscles, etc., compared with conditions in related alate taxa. Comparative research studies on groups with wing-dimorphism were presented by Lubbock (1881), Saini et al. (1982), Kozlov (1986c), Keller et al. (2014) and others. It was also shown that closely related alate and flightless groups can exhibit a hind degree of variation (Friedrich & Beutel 2008a).

The main aim of this project was to examine, document and analyze thoracic structures of the flightless representatives from BIG4 orders with different patterns of modifications of the flight apparatus. A broad spectrum of traditional (e.g. histology, hand drawing) and advanced morphological techniques (e.g. microphotography, scanning electron microscopy, computer-based 3D reconstruction) were applied. Detailed data on skeleto-muscular characters are compared with an alate individual with a close phylogenetic context. It is crucial for understanding functional configurations and interactions in insects with variously developed flight apparatus.

With the burgeoning development of cutting edge molecular techniques such as Target (TE), Anchored Hybrid Enrichment (AHE) and Next-Generation Sequencing (NGS), "Big Data" have yield impressive progress in reconstructing phylogenetic patterns in insects and the entire Arthropoda (Meusemann et al. 2010; Misof et al. 2014; Bank et al. 2017; Peters et al. 2017; Espeland et al. 2018). Significantly fewer genes and markers are vastly used building on the molecular phylogenetic studies (Mengual et al. 2008; Jordaens et al. 2015; Mengual 2015; Ståhls & Barkaloy 2017). In only few cases morphological character state matrices were combined with molecular data to infer phylogenetic relationships (e.g. Bernhard et al. 2009; Mengual et al. 2015). In recent phylogenomic studies morphological data would be obviously dwarfed by the extensive molecular data sets.

Analyses of molecular data are obviously a powerful tool to reconstruct phylogenetic patterns. However, lacking a background of well-documented morphological features, they fail to recover evolutionary changes on the phenotypic level. This includes transformations of anatomical traits linked with the flight capacity, driven by selective pressures changing with environmental conditions. This difficulty can be overcome by combining detailed morphological features, in this case related to flight adaptations, with robust phylogenetic patterns obtained with extensive molecular data sets and advanced analytical methods (e.g. Peters et al. 2014). Mapping analyses (Maddison & Maddison 2005) provide a formal and reliable approach to reconstruct character evolution linked with flight or other aspects of insect biology and evolution.

With an optimized combination of modern techniques (Friedrich et al. 2014; Wipfer et al. 2016), the acquisition of high quality morphological data is greatly facilitated, including the documentation and visualization. One aim of the present project was to demonstrate the potential of such an approach, using objects of the "BIG4", with different patterns of modification or reduction of the flight apparatus. The spectrum comprises anteromotorism (males of the Japanese winter moth, Study I), posteromotorism with simplified musculature (orectochiline whirligig beetles, Study II), anteromorism with partial wing reduction (hippoboscid swift lousefly, Study III) and complete loss of the flight ability (females of the Japanese winter moth, Study I; myrmeciinae ant workers, Study IV). These morphological data are documented in detail, together with extensive information on muscular patterns in related groups. They are discussed under phylogenetic, functional and evolutionary aspects. Evolutionary transformations in the megadiverse posteromotoric Coleoptera and their highly specialized small sistergroup Strepsiptera are discussed (Study V). The newly presented data provide broader (and more detailed) insights into the diversity of the insect thoracic structures. They illustrate different evolutionary pathways resulting from various functional or environmental backgrounds or evolutionary strategies. Combined with biomechanics, physiology and other disciplines in the future, a more complete understanding of the insect locomotion system may inspire interesting application in the field of bionics.

2. Materials and techniques

2.1. List of specimen examined

Specimens examined, described and documented with hand drawings, microphotography, SEM and 3D reconstruction:

Lepidoptera, Geometridae: *Nyssiodes lefuarius* (Erschoff, 1928): fixed in Bouin and stored in 70% ethanol.

Coleoptera, Gyrinidae: *Orectochilus villosus* (Müller, 1776): fixed in FAE (formaldehyde-acetic acid-ethanol) and stored in 70% ethanol.

Diptera, Hippoboscidae: *Crataerina pallida* (Olivier in Laterille, 1812): dried specimen.

Hymenoptera, Formicidae: *Myrmecia nigrocincta* Smith, 1858: stored in 70% ethanol.

Specimens examined for comparison:

Gyrinidae: *Heterogyrus milloti* Legros, 1953: fixed and stored in 97% ethanol.

Glossinidae: *Glossina* sp.: dried museum material.

Hippoboscidae: *Ornithoica pusilla* (Schiner, 1868); *Ornithomyiia avicularia* (Linnaeus, 1758); *Lipoptena cervi* (Linnaeus, 1758); *Melophagus ovinus* (Linnaeus, 1758): dried museum material.

Streblidae: *Nycteribosca kollari* Frauenfeld, 1855: dried museum material.

Nycteribiidae: *Eucampsopoda hyrtli* Kolenati, 1856; *Nycteribia allotopa* (Speiser, 1901); *Cyclopodia horsfieldi* de Meijere, 1899; *Stylidia hermanni* Leach, 1816: dried museum material.

2.2. Morphological techniques

Hand drawings

Specimens were manually dissected in 70% ethanol under an optical microscope equipped with an illuminator. Sclerites and body margins were drawn with full lines, margins below other sclerites with dotted lines. The figures were drawn with pencil under the microscope, scanned into the computer and finished with painting software.

Microscope type

Zeiss Stemi SV11 (Carl Zeiss Microscopy GmbH, Jena, Germany)

Illuminator type

Euromex Illuminator EK-1 light system (Euromex Microscopen BV, Arnhem, Netherlands)

Painting software

Adobe Illustrator CC (Adobe System, San José, USA)

Microscopic photography

Specimens were photographed with Keyence VH-Z20R to record the general body shape and coloration. The photos were edited for layout. Labels were added by photographic software.

Photographic software

Adobe Photoshop CC (Adobe System, San José, USA)

Critical point drying

The specimen was dehydrated in an ethanol series (from 70% to 100%) transferred into acetone and dried at the critical point.

Critical point dryer type

EmiTech K850 (Emitech Technical Services LLC, Dubai, UAE): *N. lefuarius*, *O. villosus* and *M. nigrocincta*.

Quorum E3000 (Quorum Technologies Ltd, Laughton, UK): *C. pallida*.

Scanning electron microscopy (SEM)

After critical point drying, the specimen was sputter-coated with gold or gold-palladium. Micrographs were taken with scanning electron microscope and related photographic software.

Gold sputter coater type

EmiTech K500 (Emitech Technical Services LLC, Dubai, UAE): *O. villosus* and *M. nigrocincta*.

Leica Bal-TEC SCD500 (Leica Microsystems GmbH, Wetzlar, Germany): *C. pallida*.

Scanning electronic microscopy (SEM) type

XL 30 (Philips, Amsterdam, Netherlands): *O. villosus* and *M. nigrocincta*.

TM 3000 (Hitachi High-Technologies Corp., Tokyo, Japan): *C. pallida*.

Photographic software

Scanidum (ResAlta Research Technologies, Golden, USA): *O. villosus* and *M. nigrocincta*.

Computer based 3D reconstruction

After critical point drying, specimens were scanned in a micro-CT. The thoracic segments of the specimen were reconstructed three-dimensionally using 3D reconstruction software based on the μCT-image stack.

Micro-CT type and the related parameters

Skyscan 1172 (Bruker micro-CT, Knotich, Belgium) (40 kV voltage, 250 μA current, 720 ms exposure, a rotation of 360° in steps of 0.25°, image resolution 2.2 μm): *N. lefuarius*; *C. pallida*.

Skyscan 2211 (Bruker micro-CT, Knotich, Belgium) (40 kV voltage, 320 μA current, 150 ms exposure, a rotation of 360° in steps of 0.2°): *M. nigrocincta*.

German Electron Synchrotron Facility (DESY, Hamburg, Germany): *O. villosus*.

3D reconstruction software

Amira 6.0 (Thermo Fisher Scientific Ltd, Waltham, USA): *N. lefuarius*, *O. villosus*, *C. pallida* and *M. nigrocincta*.

VGStudiomax 2.0 (Volume Graphics, Heidelberg, Germany): *N. lefuarius*, *O. villosus*, *C. pallida* and *M. nigrocincta*.

2.3. Phylogenetic analysis

Parsimony

Data were entered in a character state matrix with WinClada 1.00.08 and parsimony analyses carried out with NONA 2.0 (Nixon 1999-2002; Goloboff 1999). The uninformative characters were deleted with "Mop uninformation chars". The parsimony analysis was carried out with Ratchet (Island Hopper). The presetting was 1000 iterations. Bremer support values (Bremer 1994) were also calculated with NONA 2.0. The characters for mapping were manually mapped on the newest phylogenomic results with the function "Move branch mode".

Bayesian analysis

Bayesian analysis was performed with MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The standard model for variable morphological characters (Mkv model: Lewis 2001) was used with gamma for state frequencies and 0.1 for temperature. Four simultaneous runs of 5 million generations were conducted, each with one cold and three heated chains. Samples were drawn every 500 Markov chain Monte Carlo (MCMC) steps, with the first 25% discarded as burn-in. The run was automatically stopped then the average standard deviation of split frequencies was below 0.01.

3. Published results

Own Contribution

Study I:

Liu, S.-P., Wipfler, B., Niitsu, S. & Beutel, R.G. (2017)

The thoracic anatomy of the male and female winter moth *Nyssiodes lefuarius* (Lepidoptera: Geometridae) and evolutionary changes in the thorax of moths and butterflies. *Organisms Diversity & Evolution*

Own contribution:

Study design: Wipfler, B. & Beutel, R.G.

Morphology: 80%; Comparison: 80%; Phylogeny: 75%

Study II:

Liu, S.-P., Wipfler, B. & Beutel, R.G. (2018)

The unique locomotor apparatus of whirligig beetles of the tribe Orectochilini (Gyrinidae, Coleoptera). *Journal of Zoological Systematics and Evolutionary Research*

Own contribution:

Study design: Beutel, R.G. & **Liu, S.-P.**

Morphology: 90%; Phylogeny: 50%; Function: 80%

Study III:

Liu, S.-P., Friedrich, F., Petersen, D.S., Büsse, S., Gorb, S.N. & Beutel, R.G. (in press)

The thoracic anatomy of the swift lousefly *Cratarina pallida* (Diptera) – functional implications and characters evolution in Hippoboscoidea. *Zoological Journal of the Linnean Society*

Own contribution:

Study design: **Liu, S.-P.**, Büsse, S., Petersen, D.S. & Beutel, R.G.

Morphology: 100%; Phylogeny: 50%; Function: 70%

Study IV:

Liu, S.-P., Richter, A. & Beutel, R.G. (submitted)

The thoracic anatomy of *Myrmecia nigrocincta* workers and evolutionary transformations in Formicidae (Hymenoptera). *Arthropod Systematics & Phylogeny*

Own contribution:

Study design: **Liu, S.-P.**, Beutel, R.G. & Richter, A.

Morphology: 100%; Phylogeny: 90%; Function: 70%

Study V:

Beutel, R.G., Pohl, H.W., Yan, E.V., Anton, E., **Liu, S.-P.**, Ślipiński, A., McKenna, D. & Friedrich, F. (in press)
The phylogeny of Coleopterida (Hexapoda) – morphological characters and molecular phylogenies. *Systematic Entomology*

Own Contribution:

Study design: Beutel, R.G.
Phylogeny: 10% (Bayesian analysis)

3.1. Study I

Liu, S.-P., Wipfler, B., Niitsu, S. & Beutel, R.G. (2017)

The thoracic anatomy of the male and female winter moth *Nyssiodes lefuarius* (Lepidoptera: Geometridae) and evolutionary changes in the thorax of moths and butterflies. *Organisms Diversity & Evolution*, 17(3): 565–594.

Abstract

This manuscript compares the thoracic skeletomuscular structures of both alate male and flightless female of the geometrid winter moth *Nyssiodes lefuarius* (Lepidoptera: Geometridae). The flightless females of *N. lefuarius* have not only modified thoracic structure, but also enlarged ovaries and reduced digestive system. The specific thoracic skeletomuscular configuration in females of *N. lefuarius* compared with the other flightless female lepidopterans, supports independent evolution.

This manuscript also demonstrates the evolutionary scenario of flight ability enhancement in Lepidoptera. Thoracic skeletomuscular characters from literature are mapped on the combined phylogenetic topology from recent molecular researches. The rises of Coelolepida, Heteroneura and Ditrysia link with important changes in the thoracic system. In more advanced groups of Lepidoptera, only minor changes take place in the thoracic character system. Compared the unfolding wing base of some groups of Ditrysia with palaeopteran groups, the ancestral neopteran wing base structure and secondarily evolved palaeopteran condition are supported.

Org Divers Evol
DOI 10.1007/s13127-017-0328-4

ORIGINAL ARTICLE

ORGANISMS DIVERSITY & EVOLUTION

The thoracic anatomy of the male and female winter moth *Nyssiodes lefuarius* (Lepidoptera: Geometridae) and evolutionary changes in the thorax of moths and butterflies

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Received: 26 August 2016 / Accepted: 13 March 2017

Abstract The thoraces of males and flightless females of the geometrid winter moth *Nyssiodes lefuarius* are described, documented in detail, and compared. Morphological, functional, and evolutionary aspects of the female phenotype are discussed. The flightlessness of female *N. lefuarius* is linked not only with complex modifications of the skeletomuscular structure, especially elements of the flight apparatus, but also with greatly enlarged ovaries and a reduced gut. Compared with other flightless female lepidopterans, females of *N. lefuarius* display a specific thoracic skeletomuscular configuration, which strongly suggests independent evolution, in agreement with the phylogenetic pattern. The evolutionary scenario of flight ability enhancement in Lepidoptera is demonstrated using a combined phylogeny from recent studies based on molecular data. Thoracic skeletomuscular characters are compiled and mapped, mainly using extensive information from the literature, but also including the new morphological data obtained from the male *N. lefuarius*. Important changes in the thoracic character system are linked with the rise of Coelolepida, Heteroneura, and Ditrysia. Only minor changes take place in the character system in more advanced groups of Lepidoptera. A highly unusual feature is the secondarily stiff wing type in some groups of Ditrysia without the neopteran basal folding mechanism. The morphological background of the secondarily evolved "palaeopteran" condition is a complex of different character changes. Major problems in the reconstruction of the phylogeny are a high degree of homoplasy and missing detailed data for several crucial taxa emerging close to the root of the order.

Keywords *Nyssiodes* · Thorax · Anatomy · Flightlessness · Phylogeny · Evolution · Lepidoptera

Introduction

Geometridae are one of the most diverse families of Lepidoptera. Presently, it comprises about 21,000 described species (Minet and Scoble 1998). However, the real number is certainly much higher as suggested by recent biodiversity investigations in the Neotropics (Brehm et al. 2011). In this study, we investigate *Nyssiodes lefuarius* (Erschoff, 1872), a species of a small geometrid genus (Figs. 1 and 2). This Japanese winter moth is characterized by a highly unusual life history, with adults appearing in early spring after pupal diapause from late spring to winter (Niitsu 2001; Niitsu et al. 2014). In contrast to most geometrid species, males of *N. lefuarius* are diurnal, active in the morning hours on meadows with low grass, with a flight capacity that is distinctly better than that of other winter moth species. A conspicuous feature of *N. lefuarius* is the sexual dimorphism. The males display an unmodified and functional thoracic flight apparatus, whereas the wings of adult females are almost completely reduced. The developmental processes leading to this situation were investigated in detail by Niitsu (2001) and Niitsu et al. (2014). However, the thoracic anatomy of the adults remained largely unknown.

Lepidoptera are very popular and attractive insects. With about 160,000 described species (and an estimated total of

Electronic supplementary material The online version of this article (doi:10.1007/s13127-017-0328-4) contains supplementary material, which is available to authorized users.

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Published online: 13 April 2017

half a million; e.g., Kristensen et al. 2007), they belong to the megadiverse four holometabolan orders ("BIG4"). Considering this and the economic importance of the group, the available detailed information on the thoracic anatomy appears sparse, with very few studies based on advanced anatomical techniques. Comparative studies on the thoracic skeletal morphology were published by Weber (1924, 1928), Matsuda (1970), Brock (1971), and Minet (1991), and muscles were investigated by Ehrlich and Ehrlich (1963) (Papilionoidea) and in an entire series of studies of Kozlov (e.g., 1986a, b, 2012) (only pterothorax). Many studies were morphological treatments of single species (e.g., Nüesch 1953), without a comparative approach and phylogenetic evaluation. Others were focused on specific aspects related to the thoracic morphology and flight apparatus. The wing base structure was investigated intensively by Sharplin (1963a, b, 1964a, b), the wing shape and flight performance by Betts and Wotton (1988), the wing vestiture by Simonsen (2001, 2009) and Simonsen and Kristensen (2001), and developmental patterns of wing reduction by Niitsu (2001) and Niitsu et al. (2014). Surprisingly, the thoracic anatomy of early divergent lineages like Heterobathmiidae, Lophocoronidae, and Acanthopterotetidae is only very fragmentarily known at best, even though these taxa are obviously crucial in a phylogenetic context (e.g., Nielsen and Kristensen 1996; Wiegmann et al. 2002; Regier et al. 2015; Bazinet et al. 2016).

The first aim of our study on the thoracic morphology of *N. lefuarius* (Figs. 1 and 2) is a detailed documentation with innovative morphological techniques. A recently introduced consistent nomenclature for the musculature is applied (Friedrich and Beutel 2008, 2010; Beutel et al. 2014). The anatomical data are compared and explained with respect to functional and evolutionary aspects. The skeletomuscular modifications of the female are also compared with changes of thoracic structures of other lepidopteran flightless females and other pterygote flightless insects. Possible phylogenetic scenarios leading to secondary flightlessness in different lepidopteran groups are discussed based on the morphological evidence.

The second aim of this study is to trace the character evolution of the thoracic skeletomuscular system in Lepidoptera, with a special focus on evolutionary trends related to an enhancement of the flight performance. The flight capacity of the ancestral family Micropterigidae is modest (Imada et al. 2011), and its thoracic morphological features are significant for the inference of the ordinal groundplan (Kristensen 1998). A major trend in the evolution of the order is a successive optimization of the flight apparatus, including the capacity for high speed or long-distance migration in some cases (Beutel et al. 2014), with an enlarged mesothorax and both pterothoracic segments forming a compact unit. However, a detailed evaluation of related thoracic character modifications is still lacking. We compiled a matrix with thoracic skeletal and muscular characters based on published studies and mapped them on a combined cladogram from comprehensive phylogenetic studies (Bazinet et al. 2016; Heikkilä et al. 2015). The phylogenetic concept suggested by Bazinet et al. (2016) is closest to the results of previous morphological evaluations (Kristensen and Skalski 1998) among recent molecular studies, and the result of Heikkilä et al. (2015) was based on the most comprehensive morphological and molecular dataset.

A specific and very unusual feature we discuss based on our extensive survey of thoracic characters is the secondarily lost ability to fold back the wings in members of Papilionoidea, Bombycoidea, and Geometridae (Sharplin 1963b). Due to the comparison of the wing base structure among these three groups, also referring to the two palaeopteran orders Ephemeroptera and Odonata, we suggest a possible evolutionary scenario for this feature based on the specific characteristics of the non-folding wing type in the respective groups.

As an additional comprehensive source of information, also for future studies, we homologized the previously investigated thoracic muscles (total of 164) using a general nomenclature for neopteran insects (Friedrich and Beutel 2008, 2010; Beutel et al. 2014). The results are presented in Electronic Appendix 2 covering 5 sets of outgroups (labeled in yellow) and 12 sets of Lepidoptera.

Material and methods

The present study is based on male and female specimens of *N. lefuarius* (Erschoff, 1928) (Geometridae), which were fixed in Bouin and preserved in 70% ethanol. To facilitate the visualization of the thoracic sclerites, the thorax was manually isolated from the other body parts. The specimens were manually dissected in 70% ethanol under a Zeiss Stemi SV 11 with an additional Euromex Illuminator EK-1 lighting system. The surface hairs and scales were carefully removed with forceps. Sclerites and body margins were drawn with full lines, margins below other sclerites with dotted lines. Wings and legs were omitted, except basal elements, i.e., coxae, wing base sclerites, and basal parts of main longitudinal veins. The figures were drawn with pencil under a microscope, scanned, and finished with Adobe Illustrator CC. For digital microscopy, specimens were transferred to 100% ethanol, dried at the critical point, and photographed under a Keyence VH-Z20R. The terminology of skeletal elements follows Kristensen (2003), for muscles Friedrich and Beutel (2008, 2010) and Beutel et al. (2014), and for wing base elements Sharplin (1963a, b) and Matsuda (1970).

One male and one female specimen were scanned with a Skyscan 1172 desktop μ-computed tomograph (μ-CT) at the Functional Morphology and Biomechanics Department of

Christian-Albrechts-Universität Kiel, Germany. The scans were performed with 40 kV voltage, 250 μA current, 720 ms exposure, and a rotation of 360° in steps of 0.25°. It resulted in images with a resolution of 2.2 μm. The μ-CT data were reconstructed with FEI Amira 6.0. Segmented structures were exported as stacks of tiff files into Volume Graphics VGStudiomax 2.0, which was used for volume rendering. Final images were edited using Adobe Photoshop CC and Adobe Illustrator CC for layout and adding labels.

The thoracic character state matrix for the mapping analysis contains terminal taxa from various published studies. We mainly used thoracic skeletal characters from Heikkilä et al. (2015), Nielsen and Kristensen (1996), and Sharplin (1964b) but also surveyed characters from Matsuda (1970), Kozlov (1986a), Kristensen (2003), Kristensen and Nielsen (1979), and Leite et al. (2010a, b) as supplementary data for Micropterigidae, Agathiphagidae, Opostegidae, and Papilionidae. Muscular characters were adopted from Berlese (1909), Bharadwaj et al. (1974), Dierl (1964), Ehrlich and Davidson (1961), Ehrlich and Ehrlich (1963), Korzeev (2001), Kozlov (1986a, b, c, d, 1989, 1990, 1991, 2012), MacFarlane and Eaton (1973), Maki (1938), Mitchell and Seabrook (1970, 1971), Nüesch (1953), and Srivastava (1961, 1962). The total homologized thoracic musculature for 115 species of Lepidoptera and 8 outgroup taxa was compiled in Electronic Appendix 1 with the skeletal characters based on Heikkilä et al. (2015).

We chose the taxa for mapping the analysis of thoracic characters according to the following procedure. Firstly, we excluded duplicated characters, female characters, those with a low coverage in the literatures (e.g., prothoracic musculature), and also those with varying information in different references (e.g., Ivlm9, IIIscm2, and IIIscm4 in Papilionoidea). Secondly, if possible, we used available detailed information on skeletal and muscular features of a single species. If these were not available for a single species, we chose the most closely related and most informative pair as chimaera in a family or subfamily (one providing skeletal information, the other muscular data) (see, e.g., Beutel et al. 2011). If more than one choice with respective information was available for a taxon, we chose the one with more presumptive plesiomorphic states (e.g., more muscles, according to the trend of decreasing complexity of the thoracic musculature in Holometabola suggested by Friedrich and Beutel 2010). Thirdly, to trace the evolution of thoracic characters, we manually reconstructed a tree in WinClada ("move branch mode"), based on the phylogenetic branching pattern for non-ditrysian groups from Bazinet et al. (2016) and Ditrysia from Heikkilä et al. (2015). Uninformative characters were excluded. Finally, 87 characters (Eletronic Appendix 3) for 57 lepidopteran terminals and one trichopteran (Burrows and Dorosenko 2015; Tindall 1965) and one neuropteran outgroup (Czihak 1956) taxon (Electronic Appendix 4) were mapped on the phylogenetic topology (Fig. 3).

Abbreviations

Iax2/3 meso/metathoracic first axillary sclerite, Imp2 mesothoracic first median plate, IIax2/3 meso/metathoracic second axillary sclerite, IImp2 mesothoracic second median plate, IIIax2/3 meso/metathoracic second axillary sclerite, acp2 mesothoracic antechordal process, aes1/2/3 pr/mes/metanepisternum, alss2/3 meso/metathoracic anterolateral scutal sulcus, amwp2/3 meso/metathoracic antemedian notal wing process, an antenna, apc1/2/3 pr/mes/metanapleural cleft, aps2 mesanapleural sulcus, awp2/3 meso/metathoracic anterior notal wing process, bas2/3 meso/metabasalare, bs1/2/3 pro/meso/metabasisternum, ca2/3 meso/metathoracic chorda axillaris, ce compound eye, cld2 mesoclidium, cp3 metathoracic cubital plate, cx1 procoxa, dc1/2/3 pro/meso/metadiscrimen, ec2/3 meso/metaeucoxa, em1/2/3 pro/mes/metepimeron, es1/3 pro/metepisternum, fs1/2/3 pro/meso/metafurcasternum, fu1/2/3 profurcal arm/mesofurcal prong/primary metafurcal arm, fw forewing, hm2/3 meso/metathoracic humeral plate, hw hindwing, ip1 interpleurite, kes1/2/3 pro/meso/metakatepisternum, lc laterocervicale, lp labial palp, lpg2 mesolaterophragma, ma3 metathoracic median arm, me2/3 meso/metacoxal meron,

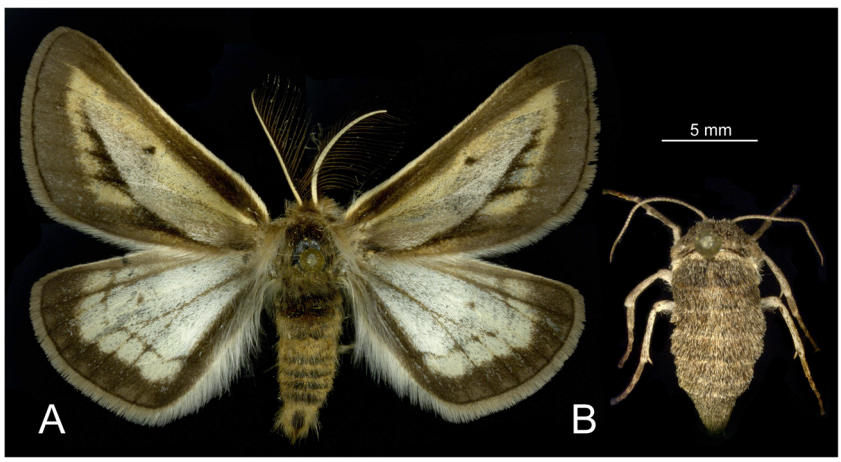


Fig. 1 *Nyssiodes lefuarius*, habitus, dorsal view. **a** Male and **b** female with reduced wings

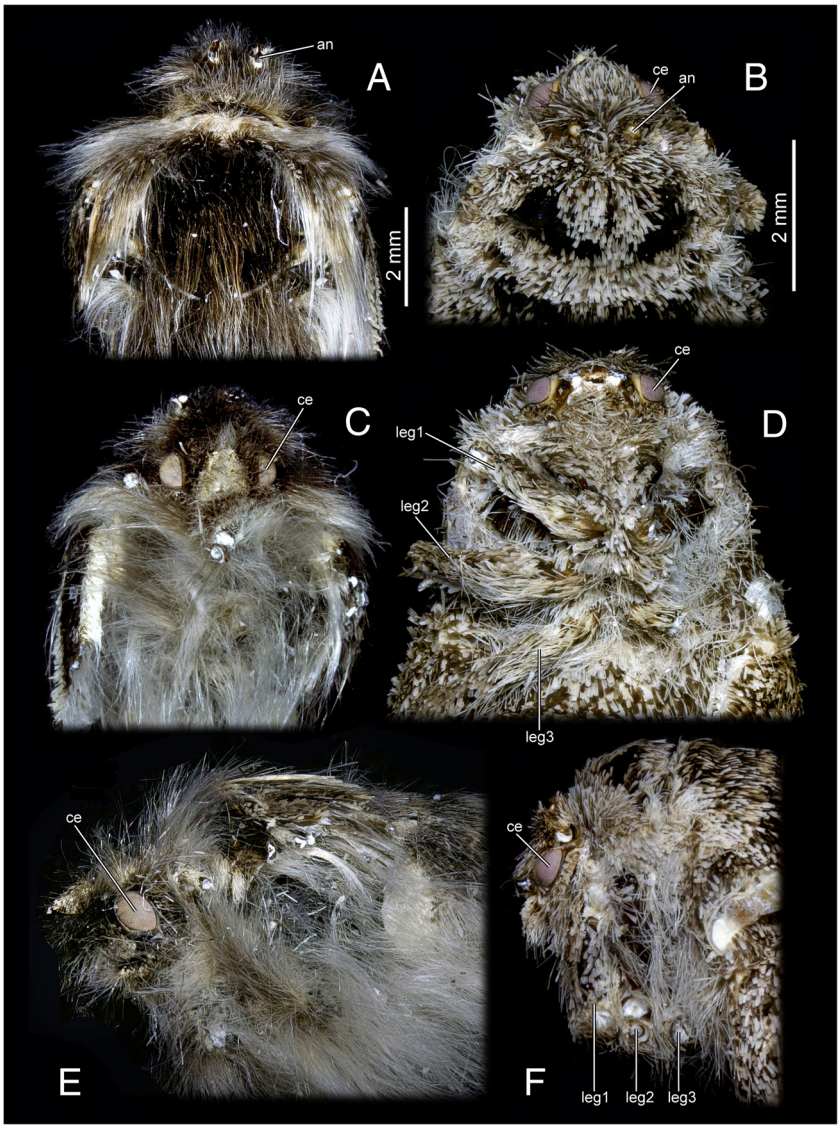


Fig. 2 *Nyssiodes lefuarius*, digital photography, head and thorax with hairs. **a** Male, dorsal view, **b** female, dorsal view, **c** male, ventral view, **d** female, ventral view, **e** male, lateral view, and **f** female, lateral view

mms2 median mesonotal sulcus, ms2/3 meso/metamarginopleural sulcus, mwp2/3 meso/metathoracic median notal wing process, nt1 pronotum, paa2 mesothoracic prealar arm, paes2 mesoparepisternum, pcs2/3 meso/metaprecoxal sulcus, pem2/3 meso/metapreepimeron, pes2 mesopreepisternum, pess2 mesoparepisternal sulcus, phg1/2 pro/mesophragma, pmwp2/3 meso/metathoracic postmedian notal wing process, pn2 mesopostnotum, ps1/2/3 pro/meso/metapleural sulcus, psc2 mesoprescutum, pscl2 mesoprescutal cleft, pwp2/3 meso/metathoracic posterior notal wing process, ptg1 patagium, rp2/3 meso/metathoracic radial plate, rss2 recurrent scutoscutellar sulcus, sa2/3 meso/metasubalare, sc2/3 meso/metascutum, scl2/3 meso/metascutellum, scp2/3 meso/metathoracic subcostal plate, sp1 1st thoracic spiracle, spa1 prospinal apodeme, sra2/3 meso/metasuralare, ss1 prospinasternum, sss2/3 meso/metascutoscutellar sulcus, ste2/3 meso/metasubtegula, tb tentorial bridge, teg2 mesotegula, tpa2/3 meso/metatergopleural apodeme, tr1/2/3 pro/meso/metatrochantin.

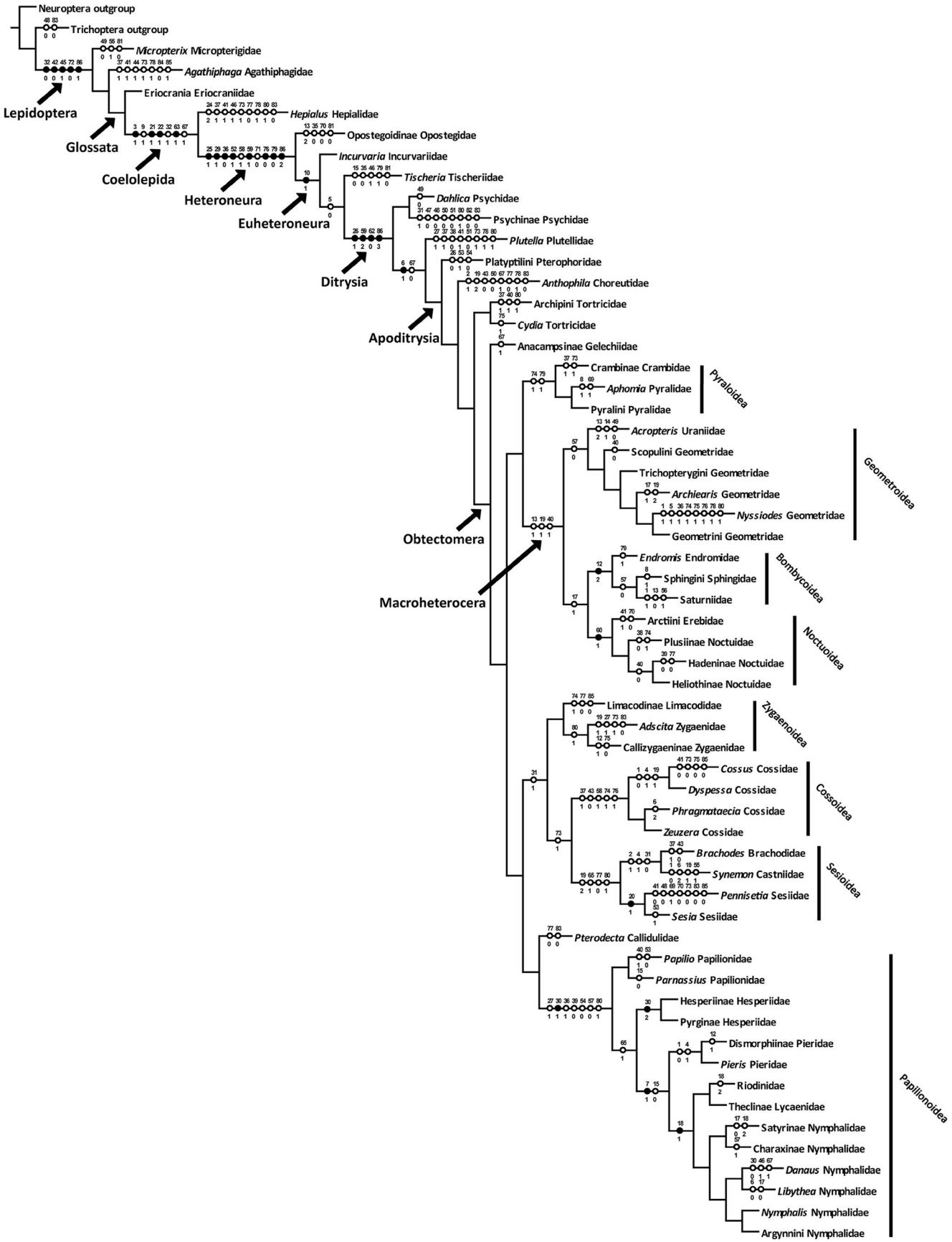
Results

Thoracic skeleton of *N. lefuarius*

Male

Cervix and prothorax The prothoracic length:width:height ratio is 2:5:5 (1:2.5:2.5). The sclerites of the prothorax are characterized by a strong degree of fusion. The small tapering posterior part of the pronotum (nt1: Fig. 4a, c) articulates with the mesoscutum. The prophragma (phg1: Fig. 5a) is suspended below this intersegmental groove. The large anterior part of the

Fig. 3 Cladogram of combined analysis of 87 lepidopteran thoracic ▶ skeletomuscular characters according to previous molecular phylogeny. Apomorphies are mapped on the tree as *circles*. Character serial number is labeled *above each circle*; status number is labeled *below each circle*. Important clades and some superfamilies are noted


Neuroptera outgroup
Trichoptera outgroup
Micropterix Micropterigidae
Agathiphaga Agathiphagidae
Eriocrania Eriocraniidae
Hepialus Hepialidae
Opostegoidinae Opostegidae
Incurvaria Incurvariidae
Tischeria Tischeriidae
Dahlica Psychidae
Psychinae Psychidae
Plutella Plutellidae
Platyptilini Pterophoridae
Anthophila Choreutidae
Archipini Tortricidae
Cydia Tortricidae
Anacampsinae Gelechiidae
Crambinae Crambidae
Aphomia Pyralidae
Pyralini Pyralidae
Acropteris Uraniidae
Scopulini Geometridae
Trichopterygini Geometridae
Archiearis Geometridae
Nyssiodes Geometridae
Geometrini Geometridae
Endromis Endromidae
Sphingini Sphingidae
Saturniidae
Arctiini Erebidae
Plusiinae Noctuidae
Hadeninae Noctuidae
Heliothinae Noctuidae
Limacodinae Limacodidae
Adscita Zygaenidae
Callizygaeninae Zygaenidae
Cossus Cossidae
Dyspessa Cossidae
Phragmataecia Cossidae
Zeuzera Cossidae
Brachodes Brachodidae
Synemon Castniidae
Pennisetia Sesiidae
Sesia Sesiidae
Pterodecta Callidulidae
Papilio Papilionidae
Parnassius Papilionidae
Hesperiinae Hesperiidae
Pyrginae Hesperiidae
Dismorphiinae Pieridae
Pieris Pieridae
Riodinidae
Theclinae Lycaenidae
Satyrinae Nymphalidae
Charaxinae Nymphalidae
Danaus Nymphalidae
Libythea Nymphalidae
Nymphalis Nymphalidae
Argynnini Nymphalidae
Lepidoptera
Glossata
Coelolepida
Heteroneura
Euheteroneura
Ditrysia
Apoditrysia
Obtectomera
Macroheterocera
Pyraloidea
Geometroidea
Bombycoidea
Noctuoidea
Zygaenoidea
Cossoidea
Sesioidea
Papilionoidea

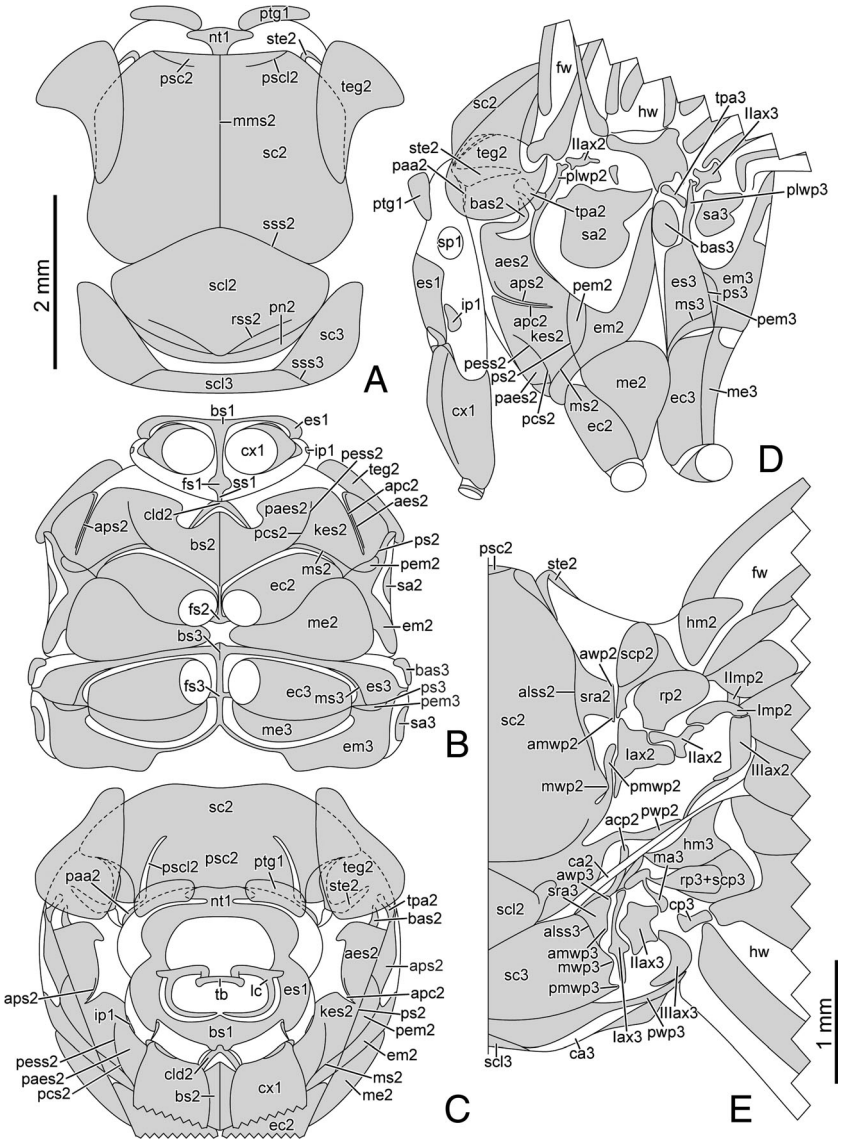


Fig. 4 *Nyssiodes lefuarius*, line drawing, male thoracic extroskeleton. **a** Dorsal view, **b** ventral view, **c** frontal view, **d** lateral view, and **e** wing base. *Scale bars*, 2 mm for (**a**)–(**d**); 1 mm for (**e**)

pronotum forms the dorsal margin of a sclerotized ring around the foramen occipitale. The patagia (ptg1: Figs. 4a, c, d, 6a, f, 7a, and 8a) are located between the anterior pronotal part and the posterior Y-shape pronotal region (Kristensen and Skalski 1998, median pronotal sclerotization). Ventrally, the pronotum is fused with the proepisternum. The laterocervicale (lc: Fig. 4c) is mesally attached to the fusion zone of the pronotum and the proepisternum by a very small process. It has a long and thin lower arm that reaches ventrally to the head capsule. Dorsally, it is fused with the tentorial bridge (tb: Fig. 4c). The proepisternum (es1: Figs. 4b–d and 6f) is an elongated sclerite of the lateral body wall. Anterodorsally, it is connected with the laterocervicale. It fuses dorsally with the pronotum and anteroventrally with the probasisternum (bs1: Fig. 4b, c), thus forming the proprecoxal bridge. Ventrally, the proepisternum forms the pleural part of the coxo-pleural joint. The coxo-pleural joint has a small narrow sclerite connected dorsally with the proepisternum and ventrally with the procoxa. The proendopleuron is invaginated along the posterior margin of the proepisternum. Near the proepisternal postero-ventral margin, a small sclerite is embedded in the pleural membrane, possibly representing the interpleurite (ip1: Figs. 4b–d and 6c). The dorsal part of the procoxa (cx1: Figs. 4b–d, 6b, c, 7a, and 8a) forms the ventral element of the coxo-pleural joint. The postero-dorsal procoxal margin is strongly concave. The probasisternum forms the ventral part of a sclerotized ring around the foramen occipitale, with a well-visible mesodorsal process between the two lower arms of the laterocervicalia. It extends postero-mesad between the procoxae and is fused posteriorly to the almost round profurcasternum (fs1: Fig. 4b). The profurcasternum is fused with the posteriorly elongated prospinasternum (ss1: Figs. 4b and 7b), and the anteriorly directed prospinal apodeme (spa1: Fig. 7a) is placed on its posterior apex. The profurcal arm (fu1: Fig. 7e) invaginated from

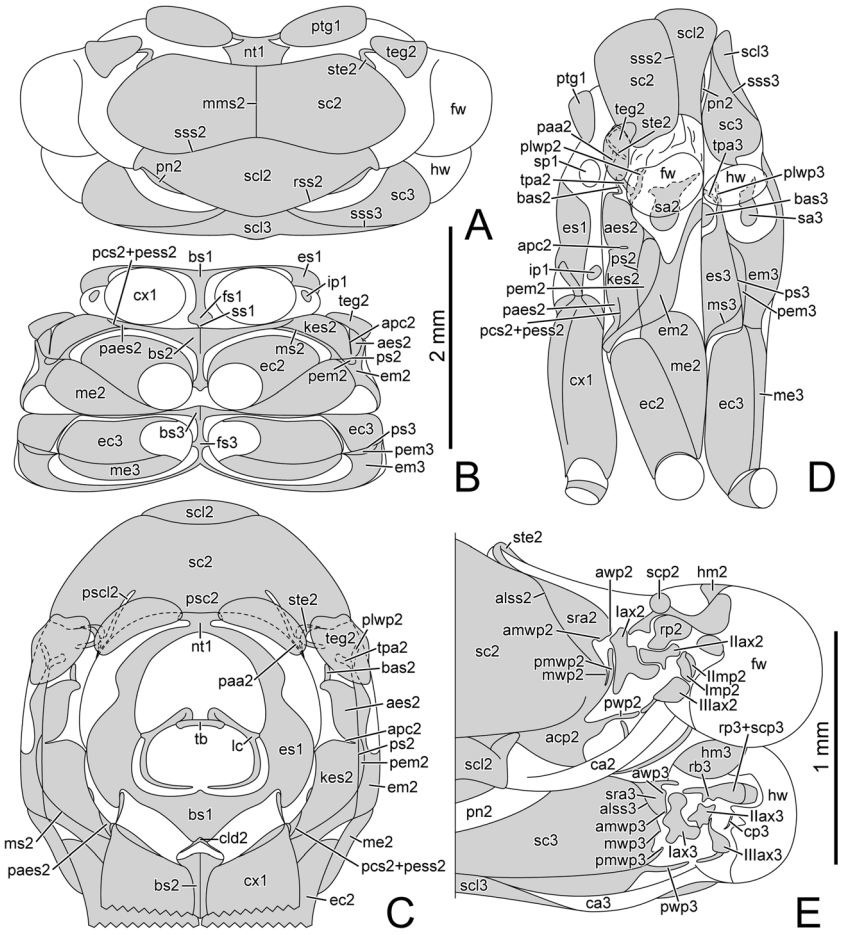


Fig. 5 *Nyssiodes lefuarius*, line drawing, female thoracic extroskeleton. **a** Dorsal view, b ventral view, **c** frontal view, **d** lateral view, and **e** wing base. *Scale bars*, 2 mm for (**a**)–(**d**); 1 mm for (**e**)

the profurcasternum is synscleritous with the lower part of the proendopleuron, both forming the profurcopleural bridge. The dorsal part of the profurcal arm forms a process. The small prodiscrimen (dc1: Fig. 7a) expands anteriorly from the basal part of the profurcal arm. The first thoracic spiracle (sp1: Fig. 4d) is embedded in the membranous area between the patagium and the proepisternum.

Mesothorax The mesothoracic length:width:height ratio is 9:8:10 (1.13:1:1.25). The large quadrate mesoscutum (sc2: Figs. 4a, c–e, 6a, e and 8a) is subdivided by a dark median mesonotal sulcus (mms2: Fig. 4a). A pair of mesoprescutal clefts (pscl2: Fig. 4a, c) delimits the anterior mesoprescutum (psc2: Fig. 4a, c, e) from the rest of the sclerite. A thin mesoprealar arm (paa2; Fig. 4c, d) is present laterally on each side. The large nearly triangular mesotegula (teg2: Figs. 4a–d, 6a, e and 8a) is laterally connected with the mesoprescutum along its anterior 2/3. A tiny strip-like mesosubtegula (ste2: Fig. 4a, c–d) attaches at the anterolateral mesoscutal margin. A sclerotized strip connects it to the mesoprealar arm. Laterally, the mesosubtegula extends beneath the mesotegula and finally connects with the mesopleural wing process below the surface membrane. The concave mesoscutoscutellar sulcus (sss2: Fig. 4a) distinctly separates the flat and pentagonal mesoscutellum (scl2: Figs. 4a, e and 6a, e) from the anterior mesonotum. The bent recurrent scutoscutellar sulcus (rss2: Fig. 4a) is strongly elevated on the posterior mesoscutellar 1/3. The following mesopostnotum (pn2: Fig. 4a) appears as a narrow strip separated from the metanotum by a wide membranous area. The very large and curved mesophragma (phg2: Fig. 7b) extends postero-ventrad reaching into the anterior lumen of abdomen. The mesolaterophragma (lpg2: Fig. 7d) near the dorsolateral corner of the mesophragma extends anteroventrad.

The mesopleura is formed by the main elements mesepisternum and mesepimeron (em2: Figs. 4b–d, 6e, and 8a), which are separated by the dorsoventrally oriented mesopleural sulcus (ps2: Figs. 4b–d and 8a), and by the sclerites associated with the wing joint. The mesanapleural cleft (apc2: Figs. 4b–d, 6e and 8a) divides the mesepisternum into a dorsal mesanepisternum (aes2: Figs. 5b–d and 6e) and a ventral mesokatepisternum (kes2: Figs. 4b–d and 6b, c). The upper margin of the cleft is strengthened by the mesanapleural sulcus (aps2: Fig. 4b–d). Anterodorsally, the mesanepisternum is fused with the curved mesobasalare (bas2: Fig. 4c, d), which extends beneath the postero-ventral part of the mesotegula. The mesopleural wing process (plwp2: Fig. 4d) extends dorsad far from the postero-dorsal part of the mesanepisternum. Distally, it forms two small heads: the anterior one articulates with the

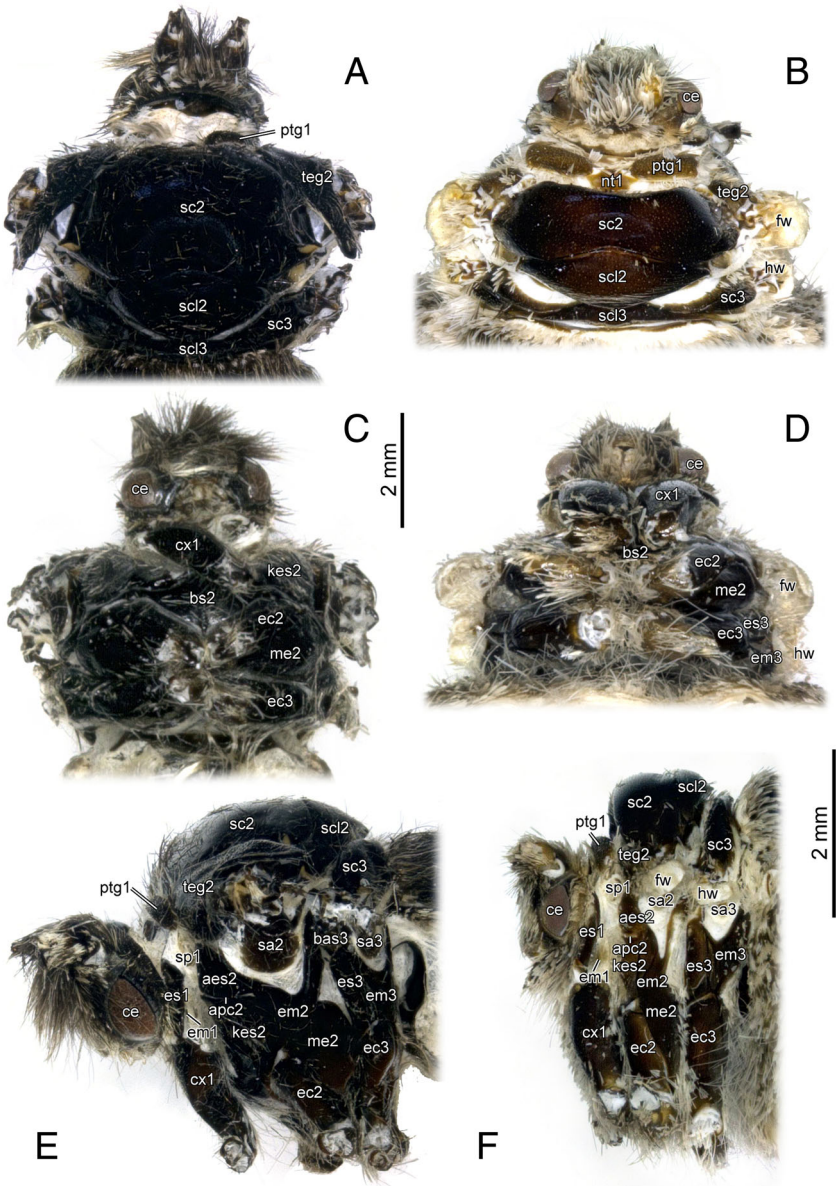


Fig. 6 *Nyssiodes lefuarius*, digital photography, head and thorax without hairs. **a** Male, dorsal view, **b** female, dorsal view, **c** male, ventral view, **d** female, ventral view, **e** male, lateral view, and **f** female, lateral view

ventral side of the subcostal plate and radial plate (namely the basal part of the subcostal and radial veins), the posterior one with the ventral side of the mesothoracic secondary axillary sclerite (IIax2: Fig. 4d). The rod-like mesotergopleural apodeme (tpa2: Fig. 4c, d) arises from the anterior side of mesopleural wing process beneath the mesotegula. Anteroventrally, the bent mesoparepisternal sulcus (pess2: Fig. 4b–d) demarcates the anteroventral mesoparepisternum (paes2: Fig. 4b–d) from the rest of mesokatepisternum, which is contiguous with the basal mesoprecoxal sulcus (pcs2: Fig. 4b–d; see Brock 1971, Fig. 7d). The mesomarginopleural sulcus (ms2: Fig. 4b–d) reaches the ventralmost part of the mesokatepisternum. It continues over the mesopleural sulcus and separates the anterior mesopreepimeron (pem2: Fig. 4b–d) from the rest of the sclerite. Postero-dorsally, the mesepimeron is strongly elongated and extends towards the lateral joint of the mesoscutellum and the mesopostnotum. The large mesosubalare (sa2: Figs. 4b, d, 6e, and 8a) lies in the membranous area between the anterior mesopleural wing process, the ventral mesepisternum and mesepimeron, and the posterior mesepipmeral elongation. It comprises a small anterior process, a large acute postero-dorsal corner and a bent ventral margin. The mesocoxa consists of the elongated anterior mesoeucoxa (ec2: Figs. 4b–d, 6c, e and 8a) and the posterior triangular mesocoxal meron (me2: Figs. 4b–d, 6c, e, and 8a), the latter synscleritous with the mesepimeron.

The mesobasisternum (bs2: Figs. 4b, c and 6c) lies medially between the mesepisterna and is fused with them. The upside-down V-shaped mesoclidium (cld2: Figs. 4b, c and 7b) continues anteriorly into the prospinasternum and extends into the anterior paired mesobasisternal grooves with its two branches. Medially, the mesobasisternum is invaginated forming a large triangular mesodiscrimen (dc2: Fig. 7a). The small

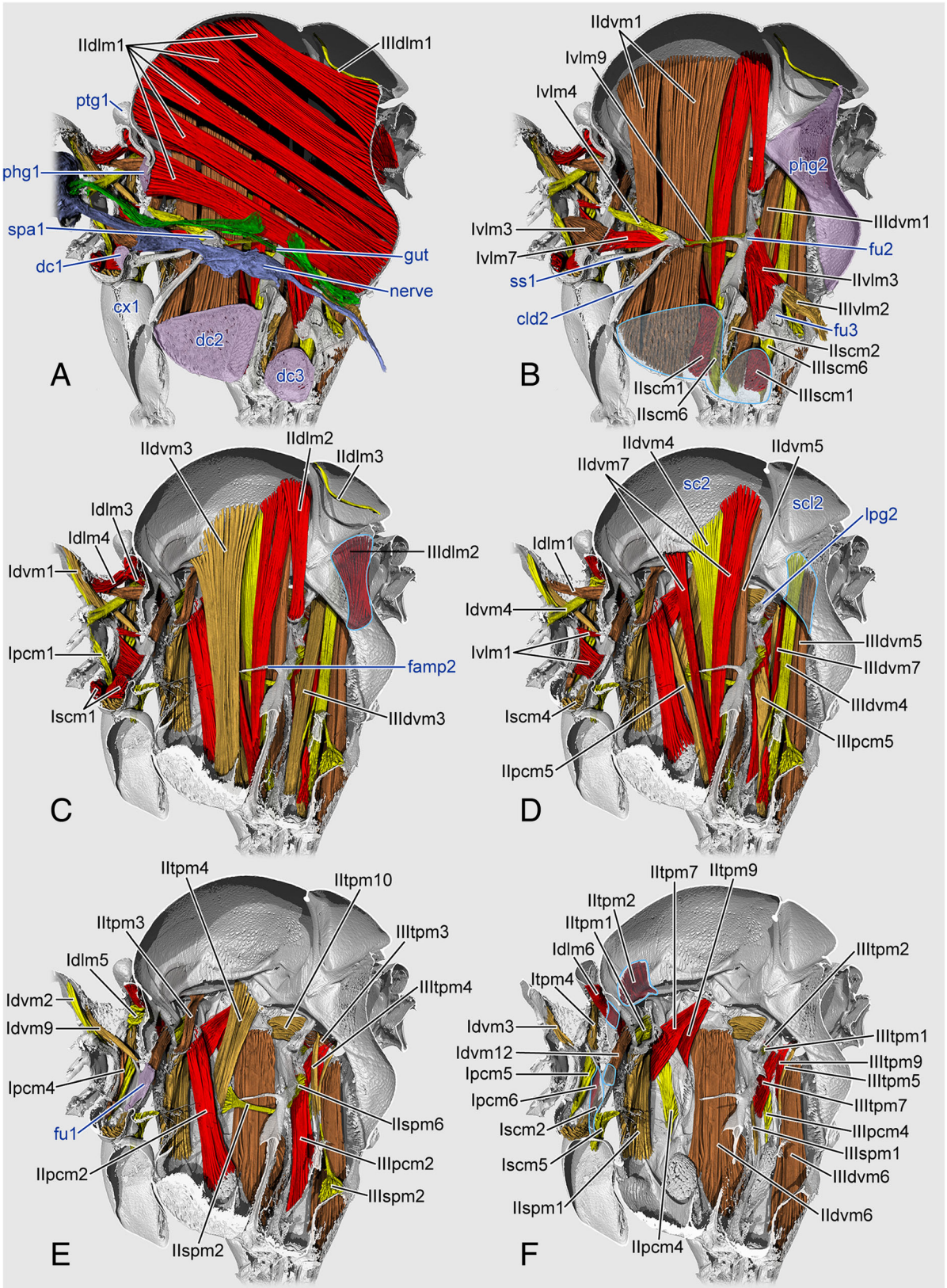


Fig. 7 *Nyssiodes lefuarius*, 3D reconstruction, male thoracic endoskeleton, and muscles. Skeletal elements in *blue lines* are rendered transparent to show the muscles behind them. Skeletal structures are labeled in *blue* and muscles in *black*. **a** Lateral view, dc1/2/3, and phg1 in *pink*; **b** lateral view and phg2 in *pink*; **c**, **d** lateral view; **e** lateral view, fu1 in *pink*; and **f** lateral view

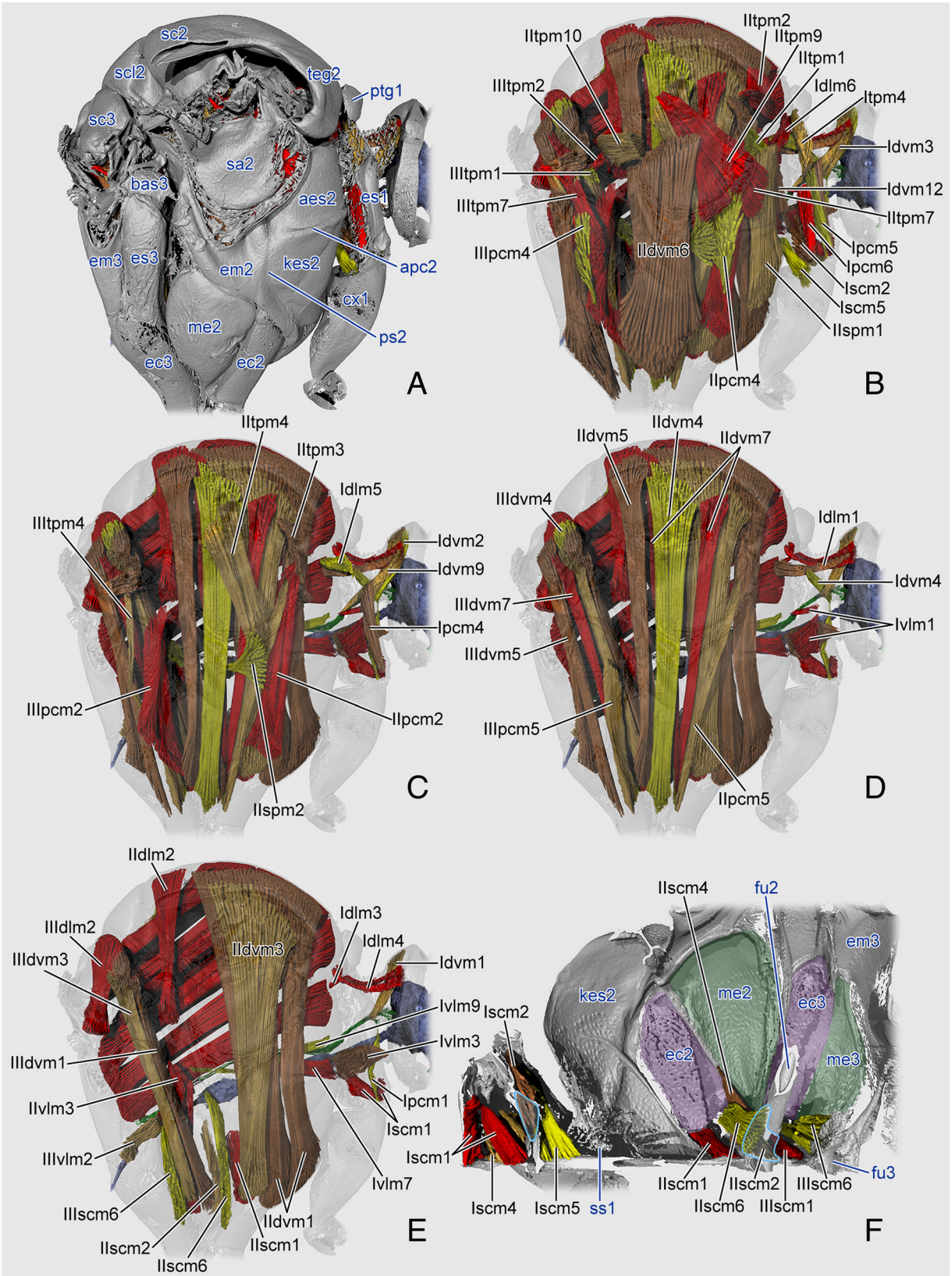


Fig. 8 *Nyssiodes lefuarius*, 3D reconstruction, male thoracic skeleton, and muscles. Skeletal elements in *blue lines* are rendered transparent to show the muscles behind them. **a** Lateral view, extraskeleton; **b**–**e** lateral view, skeleton transparentized; **f** dorsal view, sterno-coxal muscles, ec2/3 in *pink*, and me2/3 in *green*

mesofurcasternum (fs2: Fig. 4b) is fused with the thin and long mesobasisternal posterior margin and continues between the coxae. Laterally, it extends to the inner mesocoxal margins. The elongated mesofurcal prong (fu2: Fig. 7b) invaginates from the mesofurcasternum and extends laterally to the postero-dorsal mesepimeral part. Anteromedially, it bears a long and thin mesofurcal anteromedian process (famp2: Fig. 7c).

Forewing joint (Fig. 4e) The oblique anterolateral mesoscutal sulcus (alss2) delimits the lateral mesoscutal part, the mesosuralare (sra2), which includes the anterior notal wing process (awp2) and the antemedian notal wing process (amwp2). The enlarged postmedian notal wing process (pmwp2) extends anterad above the median notal wing process (mwp2). The tapering posterior notal wing process (pwp2) is a long and thin structure. The posterior antechordal process (acp2) is curved and lies beneath the long and thin membranous chorda axillaris (ca2). The large triangular first axillary sclerite (Iax2) articulates postero-proximally with the postmedian notal wing process, anteroproximally with the antemedian notal wing process, and anterodistally with the second axillary sclerite (IIax2). The latter has three distinct branches. The second axillary sclerite attaches anteroproximally to the radial plate (rp2), a large oval structure. The mesal subcostal plate (scp2) and the lateral triangular humeral plate (hm2) are located in front of the radial plate. The bent first median plate (Imp2) connects with the anterodistal margin of the second axillary sclerite, the posterior part of second median plate (IImp2), and the anterior margin of the third axillary sclerite (IIIax2). The long and thin tail of the third axillary sclerite articulates with the distal tip of posterior notal wing process.

Metathorax The metathoracic length-width-height ratio is 2:7:5 (1:3.5:2.5). The metascutum (sc3: Figs. 4a and 6a, e) is separated into two lateral plates. Their anterior margins are closely adjacent to the mesonotal postero-lateral margin. The narrow metascutellum (scl3: Figs. 4a and 6a) lies between the metascuta and is separated from them by the oblique metascutoscutellar sulci (sss3: Fig. 4a).

The vertical metapleural sulcus (ps3: Fig. 4b, d) divides the metepisternum (es3: Figs. 4b, d and 6e) and the metepimeron (em3: Figs. 4b, d and 6e). Dorsally, it is extended as the metapleural wing process (plwp3: Fig. 4d) which ends with two small heads: the anterior head articulates with the ventral side of the humeral plate and the combined radial and subcostal plate, the posterior one with the ventral side of second axillary sclerite (IIax3: Fig. 4d). The rod-like metatergopleural apodeme (tpa3: Fig. 4d) connects with the pleural wing process at half of its length. The ventral metepisternal margin is delimited by the marginopleural sulcus (ms3: Fig. 4b, d), which originates from the ventral part of the metapleural sulcus. The preepimeron (pem3: Fig. 4b, d) is separated from the remaining metepimeron by a bent sulcus. The metepimeron is extended postero-dorsal towards the metascutellar posterior margin. Postero-ventrally the metepimeron appears widened in ventral view. The oval metabasalare (bas3: Figs. 5b, d, 6e and 7a) is placed above the metepisternum and below the tergopleural apodeme. The metasubalare (sa3: Figs. 4b, d and 6e) has a rounded anterodorsal corner, a blunt postero-dorsal corner and a rounded ventral margin. It is embedded in the membranous field between the anterior metapleural wing process and the ventral and posterior metepimeron. The metacoxae comprise the elongated anterior metaeucoxa (ec3: Figs. 4b, d, 6c, e and 7a) and the narrow posterior metacoxal meron (me3: Fig. 4b, d), the latter connecting with the midpoint of ventral metapleural margin.

The narrow metabasisternum (bs3: Fig. 4b) is fused with the dorsal metepisterna. Anteromesally it bears a very small convex process, and medially it invaginates as a triangular metadiscrimen (dc3: Fig. 7a). Postero-mesally it is fused with the metafurcasternum (fs3: Fig. 4b), which is a long and slender sclerite between the metacoxae, with the very small lateral arms connecting with the inner metacoxal margins. The short and strong primary metafurcal arm (fu3: Fig. 7b) extends anterodorsal. Posteriorly the metafurcasternum divides into two secondary furcal arms and fuses laterally with the metepimeron.

Hindwing joint (Fig. 4e) The oblique mesanterolateral scutal (alss3) delimits the lateral metascutal part as the mesosuralare (sra3), which includes the anterior notal wing process (awp3) and the antemedian notal wing process (amwp3). The following median notal wing process (mwp3) is blunt, and the postmedian notal wing process (pmwp3) is small. The tapering posterior notal wing process (pwp3) is long and thin. The membranous chorda axillaris (ca3) extends along the posterior margins of the metascutum and posterior notal wing process. The proximal margin of the long and thin first axillary sclerite (Iax3) is close to the metascutal lateral margin. The pentagonal second axillary sclerite (IIax3) articulates proximally with the first axillary sclerite. The long median arm (ma3) connects with the anterodistal corner of the second axillary sclerite and is fused with the combined radial and subcostal plate (rp3 + scp3). The latter is placed between the anterior triangular humeral plate (hm3) and the posterior rectangular cubital plate (cp3, or median plate). The bent third axillary sclerite (IIIax3) articulates anteroproximally with the second axillary sclerite and posteriorly with the anterodistal margin of posterior notal wing process.

Female

For the female, we only describe the features distinguishing it from the male. The female wings (fw, hw: Figs. 5d, 6f and 10a) are strongly reduced as two ovoid structures almost completely lacking a venation. The entire female thorax is compressed in longitudinal direction. The general configuration of the sclerites is similar to the condition observed in the male, but the shape of elements is strongly modified. A large proportion of the thoracic lumen is occupied by the very large ovaries entering the

thorax from the abdomen. The gut is completely reduced in the thoracic region. The intersegmental sclerite between the thorax and abdomen is shifted forward, connecting with the primary metafurcal arm, the mesofurcal prong, the prospinal apodeme, and finally almost reaching the cervical area.

Cervix and prothorax The female prothorax is very similar to that of the male. The prothoracic length:width:height ratio is 1:5:5. The prophragma (phg1: Fig. 9b) is fused with the dorsal area of the intersegmental sclerite between the thorax and abdomen. The postero-dorsal margin (cx1: Figs. 5b–d, 6f, 9a and 10a) is not concave in lateral view.

Mesothorax The mesothoracic lumen is strongly reduced, with very limited space for the muscles. The mesothoracic length:width:height ratio is 1:2:2. The mesonotum (sc2: Figs. 5a, c–e, 6b, f and 10a) is a flat sclerite. The anterior mesoscutal part is strongly bent downwards. As a consequence, the mesoprescutum (psc2: Fig. 5c), which is delimited by the paired mesoprescutal clefts (pscl2: Fig. 5c), is positioned beneath the remaining mesoscutal part and not visible in dorsal view. The triangular mesotegula (teg2: Figs4a–d, 6b, f and 10a) is small and does not cover the lateral mesoscutal margin. The mesoscutellum (sc12: Figs. 5a, d, e, 6b, f and 10a) is flat and triangular. The recurrent scutoscutellar sulcus (rss2: Fig. 5a) connects with the mesoscutellar posterior margin, which extends posteriorly until reaching the anteromesal metanotal part. Therefore, the mesopostnotum (pn2: Fig. 5a) appears as two separate narrow elements. The small mesophragma (phg2: Fig. 9b) extends anteriorly and attaches to the posterior pronotal margin. No mesolaterophragma is present in this area. The mesanapleural cleft (apc2: Figs. 5b–d, 6f and 10a) is shorter than in the male and not supported by a mesanapleural sulcus. The mesosubalare (sa2: Figs. 5d and 6f) is narrower than in the male. The mesoclidum (cld2: Figs. 5c and 9a) and the anterior margin of the mesobasisternum (bs2: Fig. 5b) are concealed in the fold between the pro- and mesothorax, which has a similar shape as in the male. The mesofurcal prong (fu2: Fig. 5b) connects with the ventral area of the intersegmental sclerite without an anteromedian mesofurcal process.

Fore wing joint (Fig. 5e) The chorda axillaris (ca2) is thickened. The first axillary sclerite (Iax2) is shifted forward to articulate anteroproximally with the anterior notal wing process (awp2). The radial plate (rp2) connects along the entire anterior margin of the second axillary sclerite (IIax2). The subcostal plate (scp2) is round. The first and second median plates (Imp2, IImp2) are equally sized and closely connect with each other. The third axillary plate (IIIax2) is much shorter than in the male.

Metathorax The metathoracic lumen is also strongly reduced by the ovaries. The metathoracic length:width:height ratio is 1:5:4. The metascuta (sc3: Figs. 5a, d, e, 6b, f and 10a) reach further mesad than in the male. Their anteromesal edges are covered by the posterior mesoscutellar margin. The metapleural wing process (plwp3: Fig. 5d) has only one head. The margin of the posterior metepimeron (em3: Figs. 6d, f and 10a) is not enlarged in the postero-ventral area. The metabasalare (bas3: Fig. 5d) is semicircular in lateral view. The metasubalare (sa3: Figs. 5d and 6f) is narrower than in the male. The primary metafurcal arm (fu3: Fig. 9b) connects with the ventral area of the intersegmental sclerite, and its dorsal part extends laterad.

Hind wing joint (Fig. 5e) A small process of the suralare (sra3) extends laterally from its anterior margin. The chorda axillaris (ca3) is separated from the posterior notal wing process (pwp3) by a wide membranous area. The first axillary sclerite (Iax3) is short and wide and articulates with the second axillary sclerite (IIax3) at its lateral apex. The radial bridge (rb3) is reduced as two very small posterior processes of the combined radial and subcostal plate (rp3 + scp3). The humeral plate (hm3) is a large semicircular sclerotized area at the anterior margin of hind wing (hw). The cubital plate (cp3, or median plate) is very narrow. The third axillary sclerite (IIIax3) bears an anterior process articulating with the second axillary sclerite, and a long tail extending to the distal tip of posterior notal wing process.

Thoracic musculature of *N. lefuarius*

Male (Figs. 7 and 8)

Prothorax Dorsal longitudinal muscles: *Idlm1* M. prophragma-occipitalis, bent upwards: O (=origin): anterolateral area of prophragma; I (=insertion): dorsolateral area of occipitale. *Idlm3* M. prophragma-cervicalis, slender: O: dorsal-lateral area of prophragma; I: anterior margin of pronotum. *Idlm4* M. cervico-occipitalis dorsalis, bent upwards: O: anteromedian area of pronotum; I: dorsolateral area of occipitale. *Idlm5* M. pronoto-phragmalis anterior, flat: O: anterolateral margin of pronotum; I: dorsolateral area of prophragma. *Idlm6* M. pronoto-phragmalis posterior: O: median area of pronotum; I: mesoprealar arm.

Dorsoventral muscles: *Idvm1* M. cervico-occipitalis anterior: O: dorsal arm of laterocervicale; I: dorsal area of occipitale. *Idvm2* M. cervico-occipitalis medialis: O: dorsal arm of laterocervicale; I: dorsolateral area of occipitale. *Idvm3* M. cervico-occipitalis posterior, bent laterally: O: lateral part of dorsal arm of laterocervicale; I: dorsolateral area of occipitale. *Idvm4* M. pronoto-cervicalis lateralis: O: lateral inner margin of pronotum; I: tentorial bridge. *Idvm9* M. profurca-occipitalis, very narrow on profurcal arm: O: dorsal part of profurcal arm; I: dorsolateral area of occipitale. *Idvm12* M. profurca-mesonotalis: O: posterior part of profurcal arm; I: mesoprealar arm.

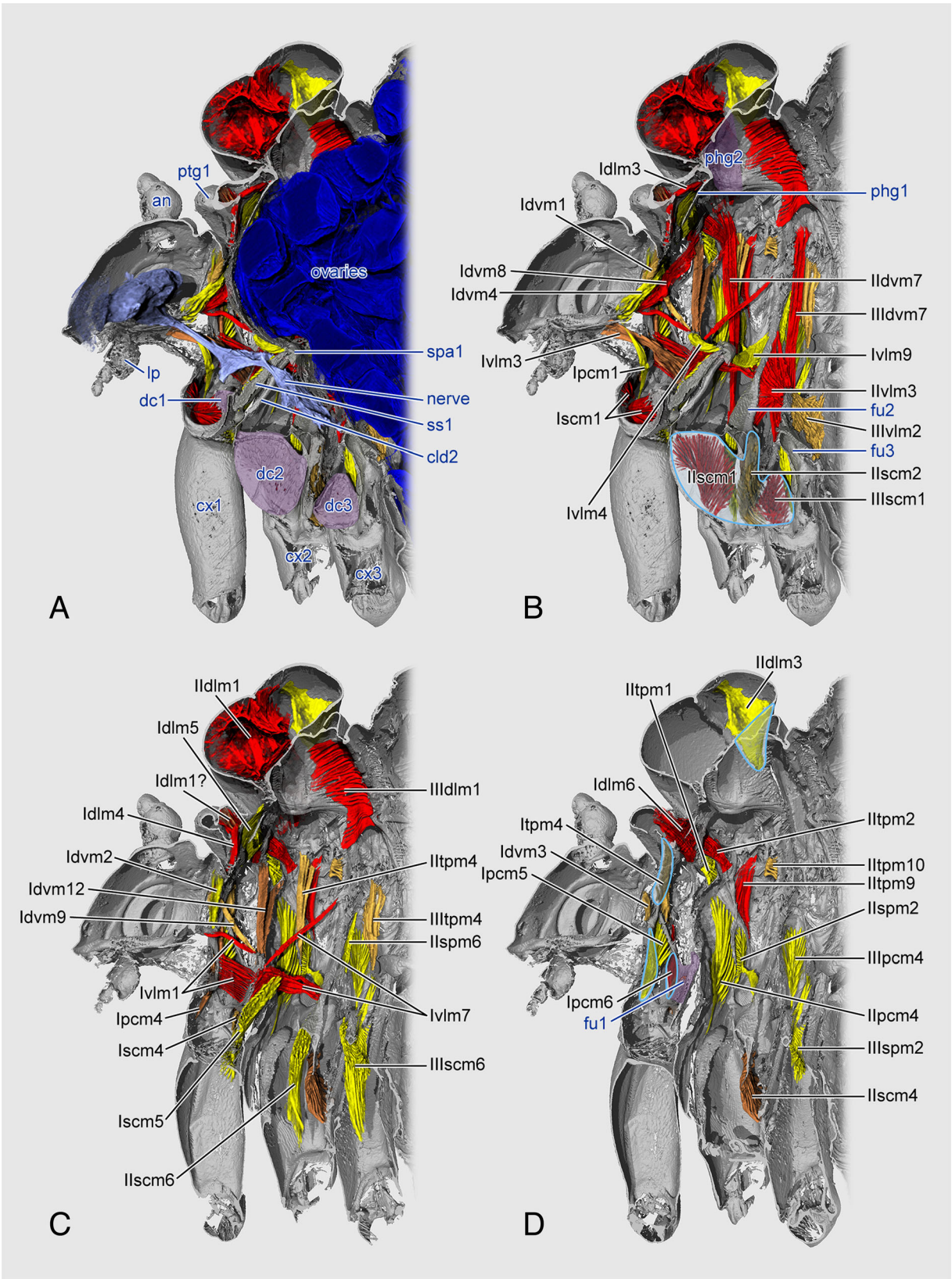


Fig. 9 *Nyssiodes lefuarius*, 3D reconstruction, female thoracic endoskeleton, and muscles. Skeletal elements in *blue lines* are rendered transparent to show the muscles behind them. Skeletal structures are labeled in *blue* and muscles in *black*. **a** Lateral view, dc1/2/3, and phg1 in *pink*; **b** lateral view, phg2 in *pink*; **c** lateral view; **d** lateral view, fu1 in *pink*

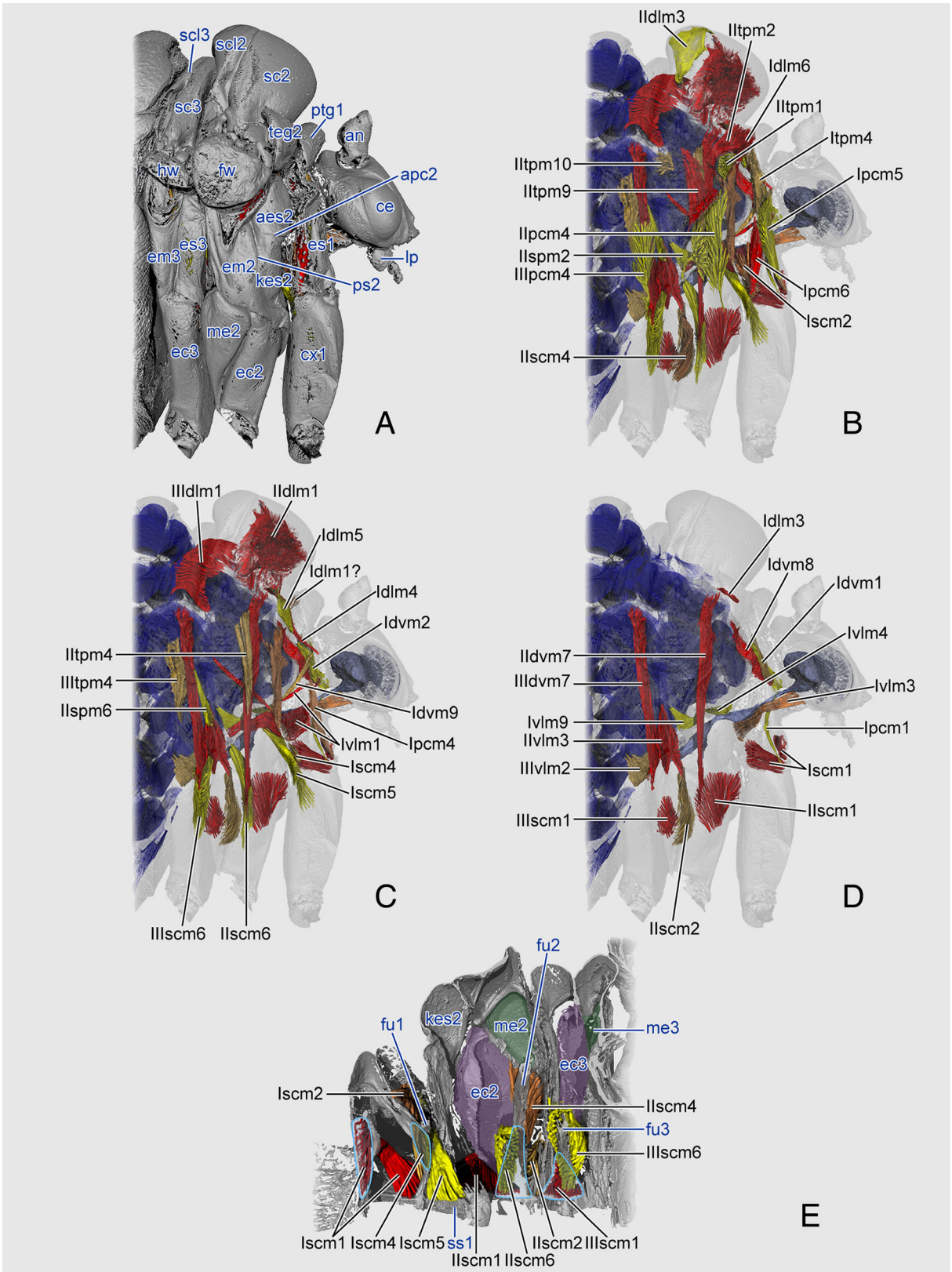


Fig. 10 *Nyssiodes lefuarius*, 3D reconstruction, female thoracic skeleton, and muscles The skelet al elements in *blue lines* are rendered transparent to show the muscles behind them. Skeletal structures are labeled in *blue*, muscles in *black*.. **a** Lateral view, extraskeleton; **b**–**d** lateral view, skeleton transparentized; **e** dorsal view, sterno-coxal muscles, ec2/3 in *pink*, and me2/3 in *green*

Tergopleural muscles: *Itpm4* M. pronoto-apodemalis anterior: O: anterolateral area of pronotum; I: postero-dorsal part of proepisternum.

Pleuro-coxal muscles: *Ipcm1* M. procoxa-cervicalis, thin and slightly bent: O: anterolateral rim of procoxa; I: tentorial bridge. *Ipcm4* M. propleuro-coxalis superior, wider on area of proepisternum, narrowing towards procoxal rim: O: dorsal area of proepisternum; I: anterolateral procoxal rim. *Ipcm5* M. propleuro-coxalis inferior, wider on area of proepisternum, narrowing towards procoxal rim: O: anterodorsal part of proepisternum; I: anterolateral procoxal rim. *Ipcm6* M. propleuro-coxalis posterior: O: postero-dorsal part of proendopleuron; I: postero-lateral procoxal rim.

Ventral longitudinal muscles: *Ivlm1* M. profurca-cervicalis, two bundles, slender dorsally, wide mesally: O: dorsal and anteroventral part of profurcal arm; I: posterior part of ventral arm of laterocervicale. *Ivlm3* M. profurca-tentorialis, conical, wider on area of profurcal arm, narrowing towards occipitale: O: anterior part of profurcal arm; I: ventral area of occipitale. *Ivlm4* M. profurca-spinalis: O: postero-dorsal part of profurcal arm; I: prospinal apodeme. *Ivlm7* M. profurca-mesofurcalis, long triangular, wider on area of profurcal arm, narrowing towards mesofurcal anteromedian process: O: postero-mesal part of profurcal arm; I: mesofurcal anteromedian process. *Ivlm9* M. prospina-mesofurcalis: O: prospinal apodeme; I: anteromedian part of mesofurcal prong.

Sterno-coxal muscles: *Iscm1* M. profurca-coxalis anterior, two bundles: O: probasisternum and prodiscrimen; I: anterior procoxal rim. *Iscm2* M. profurca-coxalis posterior, flat and triangular, wider on profurcal arm, narrower on procoxal rim: O: posterior part of profurcal arm; I: postero-lateral procoxal rim. *Iscm4* M. profurca-coxalis lateralis, flat: O: basal area of profurca; I: anterolateral procoxal rim. *Iscm5* M. prospina-coxalis: O: anterior area of prospinasternum; I: mesal procoxal margin.

Mesothorax Dorsal longitudinal muscles: *IIdlm1* M. prophragma-mesophragmalis, five parallel strong bundles: O: from prophragma to anteriormost area of mesoscutum; I: mesopostnotum and mesophragma. *IIdlm2* M. mesonoto-phragmalis: O: postero-lateral area of mesoscutum; I: mesolaterophragma. *IIdlm3* M. mesoscutello-scutellaris, slender and bent downwards: O: anterior margin of mesoscutellum; I: dorsal margin of mesopostnotum.

Dorsoventral muscles: *IIdvm1* M. mesonoto-sternalis, two strongly developed parallel bundles, posterior one larger than anterior one: O: anteromesal area of mesocutum; I: from mesobasisternum to mesodiscrimen. *IIdvm3* M. mesonoto-trochantinalis posterior, strong and flat, wider at dorsal end: O: anterolateral area of mesoscutum; I: anterior mesocoxal rim. *IIdvm4* M. mesonoto-coxalis anterior: O: lateral area of mesoscutum; I: ventral area of mesomeron. *IIdvm5* M. mesonoto-coxalis posterior: O: postero-lateral area of mesoscutum; I: posterior area of mesomeron. *IIdvm6* M. mesocoxa-subalaris, wide and strong: O: mesomeron, I: dorsal area of mesosubalare. *IIdvm7* M. mesonoto-trochanteralis, two bundles: O: mesosuralare and postero-lateral area of mesoscutum; I: mesotrochanter.

Tergopleural muscles: *IItpm1* M. prophragma-mesanepisternalis, short and wide: O: mesoprealar arm; I: posterior area of mesobasalare. *IItpm2* M. mesopleura-praealaris, fan-shaped, wide on area of mesosubtegula, narrowing towards mesotergopleural apodeme: O: mesotergopleural apodeme; I: mesosubtegula. *IItpm3* M. mesonoto-basalaris: O: mesosuralare; I: ventral area of cap-like mesobasalar apodeme. *IItpm4* M. mesonoto-pleuralis anterior: O: mesal part of mesopleural ridge; I: first mesothoracic axillary sclerite. *IItpm7* M. mesanepisterno-axillaris, triangular, wide at mesanepisternum, narrowing towards 3rd axillary sclerite: O: posterior area of mesanepisternum in front of mesopleural ridge; I: mesothoracic 3rd axillary sclerite. *IItpm9* M. mesepimeron-axillaris tertius, conical, wider on mesopleural ridge, narrowing towards third axillary sclerite: O: postero-dorsal area of mesopleural ridge; I: mesothoracic third axillary sclerite. *IItpm10* M. mesepimeron-subalaris, short and wide: O: dorsal area of mesepimeral elongation; I: posterior margin of mesosubalare.

Sterno-pleural muscles: *IIspm1* M. mesopleura-sternalis, strongly developed: O: cap-like mesobasalar apodeme; I: ventral area of mesokatepisternum. *IIspm2* M. mesofurca-pleuralis, wide on mesopleural ridge area but strongly narrowing towards mesofurcal prong: O: medio-lateral part of mesofurcal prong; I: mesal part of mesopleural ridge. *IIspm6* M. mesofurca-metanepisternalis: O: postero-median part of mesofurcal prong; I: dorsal area of metepisternum.

Pleuro-coxal muscles: *IIpcm2* M. mesobasalare-trochantinalis: O: posterior margin of cap-like mesobasalar apodeme; I: anterolateral area of mesocoxa. *IIpcm4* M. mesanepisterno-coxalis posterior, fan-shaped, wider on area of mesopleural ridge, narrowing towards mesocoxal rim: O: postero-median area of mesepisternum in front of mesopleural ridge; I: lateral mesocoxal rim. *IIpcm5* M. mesanepisterno-trochanteralis, long and slender: O: postero-dorsal area of mesanepisternum in front of mesopleural ridge; I: mesotrochanter.

Ventral longitudinal muscles: *IIvlm3* M. mesofurca-metafurcalis, two wide bundles: O: postero-median part of mesofurcal prong; I: primary metafurcal arm.

Sterno-coxal muscles: *IIscm1* M. mesofurca-coxalis anterior, a flat msucle: O: posterior area of mesodiscrimen; I: anteromesal mesocoxal rim. *IIscm2* M. mesofurca-coxalis posterior, a flat muscle, narrow on mesocoxal rim: O: basal stem of mesofurca; I: posterior mesocoxal rim. *IIscm4* M. mesofurca-coxalis lateralis, fan-shaped; O: basal stem of mesofurca; I: lateral ridge between mesoeucoxa and mesomeron. *IIscm6* M. mesofurca-trochanteralis, flat: O: ventral part of mesofurcal prong; I: mesotrochanter.

Metathorax Dorsal longitudinal muscles: *IIIdlm1* M. mesophragma-metaphragmalis, flat and wide: O: dorsal area of mesophragma; I: metaphragma. *IIIdlm2* M. metanoto-phragmalis, flat and wide: O: anterior margin of metascutum; I: metalaterophragma.

Dorsoventral muscles: *IIIdvm1* M. metanoto-sternalis: O: anterolateral margin of metascutum; I: from metabasisternum to metadiscrimen. *IIIdvm3* M. metanoto-trochantinalis posterior: O: metasuralare; I: anterior metacoxal rim. *IIIdvm4* M. metanoto-coxalis anterior: O: anterolateral area of metascutum; I: mesal metacoxal rim. *IIIdvm5* M. metanoto-coxalis posterior: O: postero-lateral area of metascutum; I: posterior area of metameron. *IIIdvm6* M. metacoxa-subalaris, flat but strongly developed: O: metameron; I: cap-like metasubalar apodeme. *IIIdvm7* M. metanoto-trochanteralis: O: lateral area of metascutum; I: metatrochanter.

Tergopleural muscles: *IIItpm1* M. mesophragma-metanepisternalis, small and conical, wider on metabasalare, narrowing towards metapleural wing process: O: anterior margin of metabasalare; I: metapleural wing process. *IIItpm2* M. metapleura-praealaris, short and slender: O: metapleural wing process; I: postero-dorsal part of mesofurcal prong. *IIItpm3* M. metanoto-basalaris, flat: O: lateral metascutal margin; I: metapleural wing process above metabasalare. *IIItpm4* M. mesonoto-pleuralis anterior, flat, wider on are of metapleural process, narrowing towards 1st axillary sclerite: O: dorsal part of metapleural process; I: metathoracic 1st axillary sclerite. *IIItpm5* M. metanoto-pleuralis medialis, slightly bent posteriorly: O: dorsal part of metapleural process; I: postero-lateral area of metascutum. *IIItpm7* M. metanepisterno-axollaris, conical, wider on are of metepisternum, narrowing towards 3rd axillary sclerite: O: median area of metepisternum; I: metathoracic 3rd axillary sclerite. *IIItpm9* M. metepimero-axillaris tertius, triangular, wider on metapleural process, narrower on 3rd axillary sclerite: O: dorsal part of metapleural process; I: metathoracic 3rd axillary sclerite.

Sterno-pleural muscles: *IIIspm1* M. metapleura-sternalis, fan-shaped, wider on area of metabasisternum: O: basal part of metabasalare; I: lateral margin of metabasisternum. *IIIspm2* M. metafurca-pleuralis, wider on area of metafurca, strongly narrowing towards metapleural process: O: basal stem of metafurca; I: ventral corner of metapleural process.

Pleuro-coxal muscles: *IIIpcm2* M. metanepisterno-trochantinalis, two bundles: O: metabasalare and dorsal margin of metanepisternum; I: anterolateral metacoxal rim. *IIIpcm4* M. metanepisterno-coxalis posterior, fan-shaped, wider on area of metepisternum, narrowing towards metacoxal rim: O: postero-median area of metepisternum in front of metapleural ridge; I: lateral metacoxal rim. *IIIpcm5* M. metanepisterno-trochanteralis: O: anteromedian area of metepisternum; I: metatrochanter.

Ventral longitudinal muscles: *IIIvlm2* M. metafurca-abdominosternalis, strong and bent downwards: O: postero-dorsal part of primary metafurcal arm; I: abdominal sternite.

Sterno-coxal muscles: *IIIscm1* M. metafurca-coxalis anterior, a flat muscle: O: posterior area of metadiscrimen; I: anteromesal metacoxal rim. *IIIscm6* M. metafurca-trochanteralis: O: ventral part of primary metafurcal arm; I: metatrochanter.

Female (Figs. 9 and 10)

Prothorax Dorsal longitudinal muscles: *Idlm1?* M. prophragma-occipitalis, short: O: anterior part of pronotum; I: anterior margin of pronotum. *Idlm3* M. prophragma-cervicalis, slender: O: dorsal part of prophragma; I: anterior margin of pronotum. *Idlm4* M. cervico-occipitalis dorsalis, slender: O: anteromedian part of pronotum; I: dorsolateral area of occipitale. *Idlm5* M. pronoto-phragmalis anterior, flat: O: anterolateral area of pronotum; I: dorsolateral area of prophragma. *Idlm6* M. pronoto-phragmalis posterior, wide: O: anteromedian area of pronotum; I: mesoprealar arm.

Dorsoventral muscles: *Idvm1* M. cervico-occipitalis anterior: O: dorsal arm of laterocervicale; I: dorsal area of occipitale. *Idvm2* M. cervico-occipitalis medialis: O: lateral part of dorsal arm of laterocervicale; I: dorsolateral area of occipitale. *Idvm3* M. cervico-occipitalis posterior, bent laterally; O: lateral part of dorsal arm of laterocervicale; I: dorsolateral area of occipitale. *Idvm4* M. pronoto-cervicalis lateralis: O: lateral inner margin of pronotum; I: mesal part of dorsal arm of laterocervicale. *Idvm8* M. prophragma-tentorialis: O: mesoprealar arm; I: dorsal arm of laterocervicale. *Idvm9* M. profurca-occipitalis, slender: O: dorsal part of profurcal arm; I: dorsolateral area of occipitale. *Idvm12* M. profurca-mesonotalis, two bundles: O: postero-dorsal part of profurcal arm; I: mesoprealar arm and anterodorsal area of mesanepisternum.

Tergopleural muscles: *Itpm4* M. pronoto-apodemalis anterior: O: anterolateral area of pronotum; I: postero-dorsal part of proepisternum.

Pleuro-coxal muscles: *Ipcm1* M. procoxa-cervicalis, slender and bent anteriorly: O: anterolateral procoxal rim; I: tentorial bridge. *Ipcm4* M. propleuro-coxalis superior, slender: O: anterodorsal part of proepisternum; I: anterolateral procoxal rim. *Ipcm5* M. propleuro-coxalis inferior, wider on proepisternum, narrower on procoxal rim: O: dorsal area of proepisternum; I: anterolateral procoxal rim. *Ipcm6* M. propleuro-coxalis posterior: O: postero-dorsal area of proendopleuron; I: postero-lateral procoxal rim.

Ventral longitudinal muscles: *Ivlm1* M. profurca-cervicalis, two bundles, slender dorsally, wide mesally: O: anterior area of profurcal arm; I: posterior area of laterocervicale. *Ivlm3* M. profurca-tentorialis: O: anterior area of profurcal arm; I: ventral area of occipitale. *Ivlm4* M. profurca-spinalis: O: dorsal part of profurcal arm; I: prospinal apodeme. *Ivlm7* M.

profurca-mesofurcalis, two bundles: O: posterior area of profurcal arm; I: median and lateral parts of mesofurcal prong. *Ivlm9* M. prospina-mesofurcalis: O: prospinal apodeme; I: anteromedian part of mesofurcal prong.

Sterno-coxal muscles: *Iscm1* M. profurca-coxalis, two bundles: O: probasisternum, base of profurca and prodiscrimen; I: anterior procoxal rim. *Iscm2* M. profurca-coxalis posterior, triangular, wider on profurca, narrower on procoxal rim: O: posterior part of profurcal arm; I: postero-lateral procoxal rim. *Iscm4* M. profurca-coxalis lateralis, conical muscle, wider on profurca, narrower on procoxal rim: O: basal area of profurca; I: postero-lateral procoxal rim. *Iscm5* M. prospina-coxalis, flat: O: prospinasternum; I: postero-lateral procoxal rim.

Mesothorax Dorsal longitudinal muscles: *IIdlm1* M. prophragma-mesophragmalis, with a cotton-like texture, apparently non-functional: O: mesoscutum; I: mesophragma. *IIdlm3* M. mesoscutello-scutellaris, flat: O: anterior margin of mesoscutellum; I: posterior margin of mesoscutellum.

Dorsoventral muscles: *IIdvm7* M. mesonoto-trochanteralis, long: O: anterolateral area of mesoscutum; I: mesotrochanter.

Tergopleural muscles: *IItpm1* M. prophragma-mesanepisternalis, short: O: mesoprealar arm; I: mesobasalare. *IItpm2* M. mesopleura-praealaris: O: mesotergopleural apodeme; I: mesosubtegula. *IItpm4* M. mesonoto-pleuralis anterior: O: middle region of mesopleural ridge; I: mesothoracic 1st axillary sclerite. *IItpm9* M. mesepimeron-axillaris tertius, triangular, wider on mesopleural ridge, narrower on mesothoracic 1st axillary sclerite: O: postero-dorsal area of mesopleural ridge; I: mesothoracic 3rd axillary sclerite. *IItpm10* M. mesepimeron-subalaris, short: O: dorsal fusion area of mesepimeral elongation and mesofurcal prong; I: posterior margin of mesosubalare.

Sterno-pleural muscles: *IIspm2* M. mesofurca-pleuralis, wide on area of mesopleural ridge, narrowing towards mesofurcal prong: O: ventral part of mesofurcal prong; I: mesopleural ridge. *IIspm6* M. mesofurca-metanepisternalis: O: median part of mesofurcal prong; I: dorsal part of metepisternum.

Pleuro-coxal muscles: *IIpcm4* M. mesanepisterno-coxalis posterior, strongly developed, fan-shaped, wider on area of mesanepisternum, narrow on mesocoxal rim: O: posterior area of mesanepisternum in front of mesopleural ridge; I: antero-lateral mesocoxal rim.

Ventral longitudinal muscles: *IIvlm3* M. mesofurca-metafurcalis, two flat bundles: O: posterior area of mesofurcal prong; I: anterior area of primary metafurcal arm.

Sterno-coxal muscles: *IIscm1* M. mesofurca-coxalis anterior, fan-shaped: O: mesodiscrimen; I: anterior mesocoxal rim. *IIscm2* M. mesofurca-coxalis posterior, flat: O: basal stem of mesofurca; I: posterior area of mesocoxa. *IIscm4* M. mesofurca-coxalis lateralis, flat: O: basal stem of mesofurca; I: lateral ridge between mesoeucoxa and mesomeron. *IIscm6* M. mesofurca-trochanteralis, strong: O: ventro-basal area of mesofurcal prong; I: mesotrochanter.

Metathorax Dorsal longitudinal muscles: *IIIdlm1* M. mesophragma-metaphragmalis, flat and wide: O: posterior area of mesophragma; I: metaphragma.

Dorsoventral muscles: *IIIdvm7* M. metanoto-trochanteralis, long: O: anterolateral area of metascutum; I: metatrochanter.

Tergopleural muscles: *IIItpm4* M. mesonoto-pleuralis anterior, triangular, wider on area of metapleural process, narrowing towards 1st axillary sclerite: O: metapleural process; I: metathoracic 1st axillary sclerite.

Sterno-pleural muscles: *IIIspm2* M. metafurca-pleuralis, wide on area of primary metafurcal arm, strongly narrowing towards metapleural process: O: lateral area of primary metafurcal arm; I: metapleural process.

Pleuro-coxal muscles: *IIIpcm4* M. metanepisterno-coxalis posterior, fan-shaped, wide on area of metepisternum, narrowing towards metacoxal rim: O: metepisternum; I anterolateral metacoxal rim.

Ventral longitudinal muscles: *IIIvlm2* M. metafurca-abdominosternalis, flat: O: posterior area of primary metafurcal arm; I: abdominal sternite.

Sterno-coxal muscles: *IIIscm1* M. metafurca-coxalis anterior, triangular, attaches on metadiscrimen: O: metadiscrimen; I: anterior metacoxal rim. *IIIscm6* M. metafurca-trochanteralis, strongly developed: O: ventral area of primary metafurcal arm; I: metatrochanter.

Comparison of the thoracic musculatures of the male and female *N. lefuarius*

The female prothoracic muscles are mostly the same as those in the male. The unusual dorsal longitudinal muscle (Fig. 9c) restricted to the pronotum is possibly homologous with the male M. prophragma-occipitalis (Idlm1: Fig. 7d), even though it has no connection to the prophragma and occipitale. The dorsoventral muscle Idvm8 (Fig. 9b) is missing in the male of *N. lefuarius*, but is present in the female like in species of many other lepidopteran groups (e.g., Eriocraniidae, Hepialidae, Psychidae, Plutellidae, Sphingidae, Saturniidae, Noctuidae, *Archierais parthenias* in Geometridae; see Electronic Appendix 1). The dorsoventral muscle Idvm12 (Fig. 7f) only attaches to the mesoprealar arm in the male, whereas an additional bundle attaches to the mesanepisternum in the female (Fig. 9c). The female ventral longitudinal muscle Ivlm7 (Fig. 9c) comprises two bundles, and one of them is directly connected with the median part of the mesofurcal prong. In contrast, the entire muscle is attached to the anteromedian mesofurcal process in the male. The other ventral longitudinal muscles of the female, Ivlm4 and Ivlm9 (Fig. 9b), are bent downwards.

In contrast to the prothorax, the pterothoracic musculature is very strongly modified in the flightless female. The mesothoracic dorsal longitudinal muscle IIdlm1 has a cotton-like texture (Fig. 9c) and no clearly separated bundles. The female dorsal

longitudinal muscle IIdlm3 (Fig. 9d) is wider and more flattened than in the male. Nearly all vertical muscles of the pterothorax are absent in the female (II/IIIdlm2, II/IIIdvm1, II/IIIdvm3–6, II/IIIspm1, II/IIIpcm2, II/IIIpcm5), except for II/IIIdvm7 and II/IIIpcm4 (Fig. 9b, d). The dorsoventral muscle IIdvm7 comprises only one bundle in the female, whereas of two are present in the male. In the mesothorax, two tergopleural muscles are absent (IItpm3, IItpm7) (Fig. 9c, d). Almost all of them are missing in the metathorax (IIItpm1–3, IIItpm5, IIItpm7, IIItpm9) except for IIItpm4 (Fig. 9c). The female ventral longitudinal muscles IIvlm3 and IIIvlm2 (Fig. 9b) are bent sideways and flat.

Thoracic apomorphies of major subgroups of Lepidoptera

For a detailed evaluation and analysis, see "Discussion."

Lepidoptera

- 32.0 (Nielsen and Kristensen 1996 [=NK96]: 18): low mesophragma index, < 0.45 (a gradual modification).
- 42.0: M. mesonoto-pleuralis posterior (IItpm6) absent (also absent in Trichoptera).
- 45.1: M. mesopleura-sternalis (IIspm1) present (also present in Trichoptera and most other groups of Holometabola).
- 72.0: M. metanoto-sternalis (IIIdvm1) absent (present in Hepialoidea and Adeloidea).
- 86.1 (Sh64 (=Sharplin 1964b): 1): Free tergopleural apodeme present.

Coelolepida

- 3.1 (NK96: 13): Placement of laterocervical posterior corner distinctly above prokatepisternum (anepisternal tooth: Kristensen 2003) or pleuro-coxal bridge (covering prokatepisternum in Acanthopteroctetidae and Lophocoronidae).
- 9.1 (NK96: 14): Articulation of anteromedian pronotal sclerite with anterodorsal proepisternal corners present (absent in Acanthopteroctetidae).
- 21.1 (NK96: 21) Anterodorsal marginal area of mesepimeron anteriorly curling, concealing upper portion of mesopleural sulcus (simple in Acanthopteroctetidae, Lophocoronidae, and Neopseustidae).
- 22.1 (Sh64: 2): Length ratio of mesothoracic postmedian notal wing process (Sharplin 1964b: median notal wing process) + 1st axillary sclerite and fore wing between 1/80 and 1/50 (gradual modification).
- 32.1 (NK96: 18): Mesophragma index high, > 0.55 (gradual modification).
- 63.1 (Sh64: 6): Median plates of hind wings replaced by cubital plates.
- 67.1 (Heikkilä et al. 2015 [=He15]: 413): Posterior tendons of metafurcal apophyses elongated caudad or dorsocaudad present (repeatedly changed in Lepidoptera, even within one family (e.g., Gelechiidae), see Electronic Appendix 1).

Heteroneura

- 25.1 (Sh64: 10): Bending cuticle in the ventral mesothoracic wing joint absent (present as vestige in Heliozelidae).
- 29.1 (NK96: 20): Transverse basisternal sutures branching off and delimiting anterior mesoclidium from posterior mesobasisternum (also present in Neopseustidae).
- 36.0: M. mesoscutello-scutellaris (IIdlm3) absent (present in Papilionoidea and some Macroheteronera, see Electronic Appendix 1).
- 52.1 (Sh64: 5): Metasubtegula (Sharplin 1964b: prescutal arm) not present as recognizable individual structure, probably fused with second basalare.
- 58.1 (Sh64: 8): Metabasalare free, with remnants of episternal stalk and/or vestigial subtegula associated with it (the distribution of character states 1 and 2 is ambiguous, but the change from state 0 to a derived condition is consistently found in Heteroneura. see Electronic Appendix 4).
- 59.1 (Sh64: 12): Insertion of M. metapleura-praealaris (IIItpm2) directly on metaprescutum or on meso/metathoracic intersegmental region.
- 71.0: M. metascutello-scutellaris (IIIdlm3) absent (present in Endromidae and Saturniidae, see Electronic Appendix 1).
- 76.0: M. metanoto-basalaris (IIItpm3) absent (variable in Cossidae).
- 79.0: M. metepimero-subalaris (IIItpm10) absent (present and variable in Cossidae and Endromidae).
- 86.2 (Sh64: 1): Contact between mesothoracic tergopleural apodeme and subtegula (Sharplin 1964b: prescutal apodeme), touching each other or loosely connected (ambiguous intermediate state).

Euheteroneura

- 10.1 (NK96: 15): Proprecoxal bridge present (present in Neopseustidae and absent in Opostegidae)

Heteroneura excl. Opostegidae and Incurvariidae

- 5.0 (NK96: 12): Proprioceptive setae on apical arm of laterocervicale present (also present in Hymenoptera and quite variable in non-ditrysian groups)

Ditrysia

- 26.1 (He15: 377): Mesoclidium V-shaped or Y-shaped (Heikkilä et al. 2015: bifurcated spinasternum).

- 59.2 (Sh64: 12): Insertion of M. metapleura-praealaris (IIItpm2) on posterior side of mesothoracic laterophragma.
- 62.0 (He15: 410): Metatrochantin absent.
- 86.3 (Sh64: 1): Tergopleural apodeme, mesotergopleural apodeme and mesosubtegula fused (mesotergopleural apodeme connected with mesopleural wing process in male *N. lefuarius*).
- Selected apomorphies of advanced ditrysian groups are listed in the following. Characters with a high degree of homoplasy are not shown.

Sesiidae

- 20.1 (He15: 384): Y-shaped sulcus on mesepimeron present.

Papilionoidea

- 30.1 (He15: 380): Mesophragma with dorsal processes.
- 39.0: M. mesonoto-basalaris (IItpm3) absent (also absent in a species of Noctuidae, see Electronic Appendix 1).

Macroheterocera

- 13.1 (He15: 379): Ventral processes of mesotegula sharp (blunt in some Bombycoidea, see electronic Appendix 1).

Noctuoidea

- 60.1 (He15: 390): Metathoracic tympanal organs present.

Discussion

Secondary flightlessness and its effects on the female skeletomuscular system

Flightlessness of females of Geometridae is usually linked with a specific set of ecological traits (Snäll et al. 2007; Wahlberg et al. 2010) and also reflects some general trends of flightlessness in pterygote insects (Roff 1986, 1990; Wagner and Liebherr 1992). The completely reduced gut in the thoracic region of females of *N. lefuarius* indicates that they consume no food. The enormously enlarged ovaries, which greatly reduce the available space for muscles in the thorax (Fig. 9a), reflect a distinctly increased allocation of resources to egg production.

The flightlessness of female *N. lefuarius* has apparently no distinct effect on the movability of the head and prolegs. The prothorax and its equipment of muscles do not differ distinctly between sexes (Figs. 7 and 9; Table 1). The reduction of the dorsal longitudinal head levator muscle Idlm1 (Kristensen 2003) in the female might be due to the limited space enclosed by the pronotum, the occipitale, and the anterodorsal part of the enlarged ovaries (Fig. 9a). Its function, retraction of the head, is accomplished by the other dorsal longitudinal muscles. The presence of dorsoventral muscle Idvm8, the additional bundles of dorsoventral muscle Idvm12, and the ventral longitudinal muscle Ivlm7 strengthen the connection between the pro- and pterothorax and compensate internal pressure resulting from the strong enlargement of the ovaries. The prospinal apodeme connecting the prospina and mesoclidium braces the anterior bottom of the ovaries; meanwhile, the ventrally bent ventral longitudinal muscles Ivlm4 and Ivlm9 probably stabilize this body region. The prophragma extends anteroventrally as a part of the dorsal cover of the ovaries. In contrast to males, it is no longer an attachment site of the very strong mesothoracic dorsal longitudinal principal wing depressor muscle IIdlm1 (Kristensen 2003).

The compression of the female thorax in longitudinal direction is a general phenotype in brachypterous lepidopterans with a distinctly enlarged abdomen (Snäll et al. 2007; Wahlberg et al. 2010). This is conform with a general trend in wing-dimorphic insects, in which female body parts involved in reproduction are enlarged and those related to flight are reduced (Roff 1986). The backward shift of the recurrent scutoscutellar sulcus is likely related to the female flightlessness, as this structure normally facilitates the deformation of the tergite during the wing stroke in flying insects (Brodsky 1994). The female mesophragma is extended forward and forms a part of the dorsal cover of the ovaries. This modification impedes the normal function of the dorsal longitudinal indirect flight muscle IIdlm1, which is placed below the shortened mesoscutum (Fig. 9c). The muscle is still present but obviously degenerated in females of *N. lefuarius*, with a cotton-like texture. The reduction of this principal wing depressor (Brodsky 1994; Kristensen 2003) is a common feature in flightless insects (Wipfler et al. 2014). The modified pleural wing process with only one head is probably correlated with the disposable function as wing fulcrum, which forms the articulation with the ventral side of the wing base in insects capable of flight (Brodsky 1994). The deformation of the wing base sclerites, which play an essential role in transmitting force from the thoracic main body to the wing pairs (Brodsky 1994), is apparently due to reduced functional constraints resulting from the wing reduction. The modified shapes of the laterally bent mesofurcal prong and primary metafurcal arm apparently help to support the bottom of the ovaries, together with the prospinal apodeme. The lost anteromedian mesofurcal process possibly results from the short distance between the profurcal arm and mesofurcal prong in the compressed thorax of the female. A sclerotized elongation as attachment site of the ventral longitudinal muscle Ivlm7 is not required any more.

Table 1 Musculatures of male and flightless female lepidopteran species (present with "+" or muscular name in green, absent with "–" in orange, uncertain with "?," or "/" in yellow)

Muscle Name	*N. lefuarus*		*O. brumata* (Kozlov 1986c)		Psychinae (Dierl 1964)	
	Male	Female	Male	Female	Male	Female
Idlm1	+	+?	/	/	Lbdlm1	Lbdlm1
Idlm2	-	-	/	/	-	-
Idlm3	+	+	/	/	-	-
Idlm4	+	+	/	/	-	-
Idlm5	+	+	/	/	Idlm1	-
Idlm6	+	+	/	/	-	-
Idvm1	+	+	/	/	Lbism3	-
Idvm2	+	+	/	/	-	-
Idvm3	+	+	/	/	-	-
Idvm4	+	+	/	/	-	-
Idvm5	-	-	/	/	-	-
Idvm6	-	-	/	/	-	-
Idvm7	-	-	/	/	-	-
Idvm8	-	+	/	/	Lbism1	Lbism1?
Idvm9	+	+	/	/	Lbism2	-
Idvm10	-	-	/	/	-	-
Idvm11	-	-	/	/	-	-
Idvm12	+	+	/	/	-	-
Idvm13	-	-	/	/	-	-
Idvm14	-	-	/	/	-	-
Idvm15	-	-	/	/	-	-
Idvm16	-	-	/	/	-	-
Idvm17	-	-	/	/	-	-
Idvm18	-	-	/	/	-	-
Idvm19	-	-	/	/	-	-
Itpm1	-	-	/	/	-	-
Itpm2	-	-	/	/	-	-
Itpm3	-	-	/	/	-	-
Itpm4	+	+	/	/	Idvm1	Idvm1
Itpm5	-	-	/	/	Iism	-
Itpm6	-	-	/	/	-	-
1ppm1	-	-	/	/	-	-
1ppm2	-	-	/	/	-	-
Ispm1	-	-	/	/	-	Ipm2
Ispm2	-	-	/	/	-	-
Ispm3	-	-	/	/	-	-
Ispm4	-	-	/	/	-	-
Ispm5	-	-	/	/	-	-
Ispm6	-	-	/	/	-	-
Ispm7	-	-	/	/	-	-

The function of the widened dorsal longitudinal muscle IIdlm3 in the female is quite unclear. It is conceivable that it stabilizes the dorsal mesothoracic region. The absence of a series of dorsoventral muscles II/IIIdvm1 and II/IIIdvm3–5 and the dorsal longitudinal muscles II/IIdlm2 is apparently correlated with the lost flight capacity. They function as indirect wing levators in flying insects (Brodsky 1994). The reduction of II/IIIdvm1 is also found in all studied flightless polyneopteran species (Wipfler et al. 2014). IIdvm1 is absent in non-archostematan Coleoptera (Friedrich and Beutel 2010), apparently correlated with the reduced size of the mesothorax and posteromotorism in this group. The posterior bundle of IIdvm1 is also reduced in the flightless female of the geometrid species *Operophtera brumata* (Kozlov 1986c). The function of IIIdvm1, which is missing in most non-ditrysian lepidopterans, is not well understood according to Kristensen (2003).

Table 1 (continued)

Ipcm1	+	+	/	/	Lbism4	-
Ipcm2	-	-	/	/	-	-
Ipcm3	-	-	/	/	-	-
Ipcm4	+	+	/	/	-	-
Ipcm5	+	+	/	/	Ipm1	Ipm1
Ipcm6	+	+	/	/	-	-
Ipcm7	-	-	/	/	-	-
Ipcm8	-	-	/	/	-	-
Ivlm1	+	+	/	/	Lbvlm2&Ivlm3	-
Ivlm2	-	-	/	/	-	-
Ivlm3	+	+	/	/	Lbvlm1	Lbvlm1&Lbvlm2
Ivlm4	+	+	/	/	Ivlm2	-
Ivlm5	-	-	/	/	-	-
Ivlm6	-	-	-	-	-	-
Ivlm7	+	+	Is13	Is13	Ivlm1	Ivlm1
Ivlm8	-	-	-	-	-	-
Ivlm9	+	+	IIs12	IIs12	IIvlm3	
Iscm1	+	+	/	/	Ibm1&Ibm2?	Ibm1&Ibm2
Iscm2	+	+	/	/	-	-
Iscm3	-	-	/	/	-	-
Iscm4	+	+	/	/	-	-
Iscm5	+	+	/	/	Ibm3	-
Iscm6	-	-	/	/	Ibm4	Ibm3
Iscm7	-	-	/	/	-	-
IIdlm1	+	+	IIt14	IIt14	IIdlm1	IIdvm1
IIdlm2	+	-	IIt12	IIt12	IIdlm2	-
IIdlm3	+	+	-	-	-	-
IIdvm1	+	-	IIt-s12/13	IIt-s12	IIdvm1+2	-
IIdvm2	-	-	-	-	-	-
IIdvm3	+	-	IIt-ti2	-	IIdvm3	-
IIdvm4	+	-	IIt-cx7	IIt-cx7	IIdvm5	-
IIdvm5	+	-	IIt-cx7	IIt-cx7	IIdvm5	-
IIdvm6	+	-	IIp-cx10	IIp-cx10	IIpm8	-
IIdvm7	+	+	IIt-tr1	IIt-tr1	IIdvm4	-
IIdvm8	-	-	-	-	-	-
IIdvm9	-	-	-	-	-	-
IItpm1	+	+	IIt-p3	IIt-p3	IIpm3	-
IItpm2	+	+	IIt-p4	IIt-p4	IIpm4	-
IItpm3	+	-	IIt-p7	IIt-p7	IIpm5	IIdvm2?
IItpm4	+	+	-	-	-	-
IItpm5	-	-	-	-	-	IIdvm3?

Some direct wing muscles attached to the basalare at the wing base probably function as indirect wing levators during wing strokes (Brodsky 1994). This applies to the sterno-pleural muscles II/IIIspm1 as suggested by Friedrich and Beutel (2008), and possibly also to the pleuro-coxal muscles II/IIIpcm2 and II/IIIpcm5. IIspm1 is absent in most studied flightless polyneopterans and holometabolans (Friedrich and Beutel 2010; Wipfler et al. 2014), and IIpcm2 is missing in Siphonaptera (Friedrich and Beutel 2010). Meanwhile, their underlying secondary functions like adduction and pronation, especially involving the basalare (Brodsky 1994), should be noticed. The subalare muscles II/IIIdvm6 are supinators and accessory depressors, and their importance in flight control has been demonstrated in a species of Sphingidae (Brodsky 1994). It is interesting that the tergopleural muscle IItpm9, which directly controls the activities of the 3rd axillary sclerite during wing pronation and folding and is an important component of a functional flight apparatus (Sharplin 1964a; Brodsky 1994; Wipfler et al. 2014), is still present in female *N. lefuarius*. In contrast, the other tergopleural muscles IIItpm1 and IIItpm2

Table 1 (continued)

IItpm6	-	-	-	-	-	-
IItpm7	+	-	IIt-p13	IIt-p13	IIpm6	-
IItpm8	-	-	-	-	-	-
IItpm9	+	+	IIt-p14	IIt-p14	IIpm7	-
IItpm10	+	+	IIp6	IIp6	-	-
IItpm11	-	-	-	-	-	-
IItpm12	-	-	-	-	-	-
IIppm1	-	-	-	-	-	-
IIppm2	-	-	-	-	-	-
IIspm1	+	-	IIp3	IIp3	IIpm1	-
IIspm2	+	+	IIp-s1	IIp-s1	IIzm	IIpm2
IIspm3	-	-	-	-	-	-
IIspm4	-	-	-	-	-	-
IIspm5	-	-	-	-	-	-
IIspm6	+	+	IIp-s3	IIp-s3	-	-
IIspm7	-	-	-	-	-	-
IIspm8	-	-	-	-	-	-
IIpcm1	-	-	-	-	-	-
IIpcm2	+	-	IIp-cx3	IIp-cx3	-	IIpm1
IIpcm3	-	-	-	-	-	-
IIpcm4	+	+	IIp-cx4	IIp-cx4	-	-
IIpcm5	+	-	IIp-tr2	IIp-tr2	IIpm2	-
IIpcm6	-	-	-	-	-	-
IIvlm1	-	-	-	-	-	-
IIvlm2	-	-	-	-	-	-
IIvlm3	+	+	IIs13	IIs13	IIvlm1&IIvlm2	IIvlm1&IIvlm2
IIvlm4	-	-	-	-	-	-
IIvlm5	-	-	-	-	-	-
IIvlm6	-	-	-	-	-	-
IIvlm7	-	-	-	-	-	-
IIscm1	+	+	-	-	IIbm1	IIbm1&IIbm2
IIscm2	+	+	IIs-cx6	IIs-cx6	-	-
IIscm3	-	-	-	-	-	-
IIscm4	+	+	IIs-cx2	IIs-cx2	-	IIbm3
IIscm5	-	-	-	-	-	-
IIscm6	+	+	IIs-tr1	IIs-tr1	IIbm2	IIbm4
IIscm7	-	-	-	-	-	-
IIIdlm1	+	+	IIIt14	IIIt14	IIIdlm1	IIIdlm1&3
IIIdlm2	+	-	IIIt12	-	IIIdlm2	IIIdlm2?

and II/IIItpm3 that mainly stabilize the lateral thoracic wall (Kozlov 1986c; Brodsky 1994) are absent. Tergopleural muscles II/IIItpm3 and IIItpm5 have been shown to play a role in winged Holometabola (Friedrich and Beutel 2010), but this is not verified in Polyneoptera (Wipfler et al. 2014). Muscles II/IIItpm7 are additional wing flexors besides II/IIItpm9 (Kozlov 1986c). They are generally absent in flightless polyneopteran and holometabolan species but also in many alate representatives of Polyneoptera (Friedrich and Beutel 2010; Wipfler et al. 2014). According to Brodsky (1994), this could be related to a flat wing folding pattern as in Plecoptera.

The general absence of the large indirect wing levators in the female *N. lefuarius* is probably also due to the limited thoracic lumen caused by the very large ovaries. This is suggested by the presence of most of them in the flightless female geometrid *O. brumata* (Table 1), which has a similar thoracic lumen as the male (Fig. b in Kozlov 1986c). The laterally bent ventral longitudinal muscles IIvlm3 and IIIvlm2 in the female probably provide flexible mechanical support for the ovaries, together with the furcal arms. The potential of pterothoracic muscles to fulfill different functions (Brodsky 1994) is probably reflected by their presence in both flying and flightless insects. The dorsoventral muscles II/IIIdvm7, which are also important indirect wing muscles in flying insects (Brodsky 1994), are largely retained in the female

Table 1 (continued)

IIIdlm3	-	-	-	-	-	-
IIIdvm1	+	-	IIIt-s12	-	IIIdvm1	-
IIIdvm2	-	-	-	-	-	-
IIIdvm3	+	-	IIIt-ti2	-	IIIdvm2	-
IIIdvm4	+	-	IIIt-cx7	IIIt-cx7	IIIdvm4	-
IIIdvm5	+	-	IIIt-cx7	IIIt-cx7	IIIdvm4	-
IIIdvm6	+	-	IIIp-cx10	IIIp-cx10	IIIpm6	-
IIIdvm7	+	+	IIIt-tr1	IIIt-tr1	IIIdvm3	-
IIIdvm8	-	-	-	-	-	-
IIItpm1	+	-	IIIt-p3	IIIt-p3	IIIpm3	-
IIItpm2	+	-	-	-	-	-
IIItpm3	+	-	-	-	-	IIIdvm1?
IIItpm4	+	+	-	-	-	-
IIItpm5	+	-	-	-	-	IIIdvm2?
IIItpm6	-	-	-	-	-	-
IIItpm7	+	-	IIIt-p13	IIIt-p13	IIIpm4	-
IIItpm8	-	-	-	-	-	-
IIItpm9	+	-	IIIt-p14	IIIt-p14	IIIpm5	-
IIItpm10	-	-	-	-	-	-
IIItpm11	-	-	-	-	-	-
IIItpm12	-	-	-	-	-	-
IIIppm1	-	-	-	-	-	-
IIIppm2	-	-	-	-	-	-
IIIspm1	+	-	IIIp2	IIIp2	IIIpm1	-
IIIspm2	+	+	IIIp-s1	IIIp-s1?	IIIzm	IIIzm
IIIspm3	-	-	-	-	-	-
IIIspm4	-	-	-	-	-	-
IIIspm5	-	-	-	-	-	-
IIIpcm1	-	-	-	-	-	-
IIIpcm2	+	-	IIIp-cx3	IIIp-cx3	-	IIIpm1
IIIpcm3	-	-	-	-	-	-
IIIpcm4	+	+	IIIp-cx1	IIIp-cx1	-	-
IIIpcm5	+	-	IIIp-tr2	IIIp-tr2	IIIpm2	-
IIIpcm6	-	-	-	-	-	-
IIIpcm7	-	-	-	-	-	-
IIIvlm1	-	-	-	-	-	-
IIIvlm2	+	+	IIIs20	IIIs20	IIIvlm1&IIIvlm2	-
IIIvlm3	-	-	-	-	-	-
IIIscm1	+	+	-	-	IIIbm1	IIbm2
IIIscm2	-	-	IIIs-cx6	IIIs-cx6	-	-
IIIscm3	-	-	-	-	-	-
IIIscm4	-	-	-	-	-	-
IIIscm5	-	-	-	-	-	-
IIIscm6	+	+	IIIs-tr1	IIIs-tr1	IIIbm2	IIIbm4

N. lefuarius with only one missing bundle. This is likely due to their dual function also including leg movements. The presence of several tergopleural muscles IItpm1, IItpm2, IItpm10, and II/IIItpm4 in both males and females of *N. lefuarius* might be due to their double function of controlling the wing base sclerites and stabilizing the dorsolateral thoracic region (Kozlov 1986c; Brodsky 1994). Other flight-related skeletomuscular structures might just be retained as relicts in the female *N. lefuarius*. This suggests a relatively young age of evolutionary events leading to the loss of the flight capacity, with a taxon specific low evolutionary rate or specific developmental modalities. This probably applies to the vestigial wings, wing base sclerites and the presence of the tergopleural muscle IItpm9 in female *N. lefuarius*. In addition, it is conceivable that the locomotion restricted to walking and the reduced digestive system could also have some effects on the skeletomuscular structure of the female of *N. lefuarius*.

This probably applies to the increased size of some sternocoxal muscles. However, a quantitative comparison of muscular size is required for a precise assessment.

The pterothoracic musculature of females of *N. lefuarius* differs very distinctly from that of the male, whereas it is very similar in both sexes of *O. brumata* (compared with the male, only IIIdlm2, IIIdvm1, and II/IIIdvm3 are absent in females; Table 1). This difference reflects the frequent independent evolution of female flightlessness in Geometridae, usually in this family correlated with a specific set of ecological traits, the "winter moth syndrome" (Wahlberg et al. 2010). The programmed cell death resulting in wing reduction in the development of female *N. lefuarius* is probably a specific feature of Geometridae (Niitsu 2001; Niitsu et al. 2014) but more evidence on other groups with winglesss pecies is required for a reliable assessment. Some characters also occur in other flightless groups of Lepidoptera and other insect orders (e.g., reduction of phragmata), apparently as a result of convergent evolution affected by different genetic regulatory networks and developmental processes. Multiple origins of geometrid female flightlessness (even within a single subfamily) were shown by molecular phylogenetic studies, based on the premise of unlikely events of reversal (Snäll et al. 2007; Wahlberg et al. 2010). The very complex modifications in the female *N. lefuarius* involve not only different elements of the thoracic skeletomuscular system, but also the genital apparatus (ovaries) and digestive tract (gut reduction). This complex set of morphological changes implies irreversibility of female flightlessness in *N. lefuarius*. This is in agreement with Snäll et al. (2007) and Wahlberg et al. (2010), who suggested that reversal could be excluded if secondary flightlessness is linked with complex changes in the body composition. The evolution of wingless females in Psychidae, where even the homology of muscles is often very uncertain (Dierl 1964; Table 1), obviously has a very different evolutionary background than in Geometridae. Some thoracic muscles in female psychids have probably completely changed their original function. This likely applies to the presumptive tergopleural muscles II/IIItpm3 and II/IIItpm5 in the highly specialized worm-like females. Finally, the initial morphological setting preceding flightlessness should be taken into consideration in this context (Wipfler et al. 2014). Some seemingly flight-related structural changes might in fact be related to other evolutionary tendencies or due to phylogenetic restraints. This is obviously the case if similar features also occur in closely related taxa capable of flight. An intraspecific example would be the loss of the tergopleural muscles IIItpm2–4 in both male and female of *O. brumata* (Kozlov 1986c; Table 1). A careful comparison between studied flightless insects and their closest relatives capable of flight as a reference is necessary to avoid misinterpretations.

In summary, flightlessness of female *N. lefuarius* involves complex modifications, not only affecting flight-related skeletomuscular elements but also other structures and organs. Thoracic modifications in the female *N. lefuarius* differ from conditions occurring in other flightless female lepidopterans, thus suggesting independent evolution. Obviously, more investigations are required for a better understanding of evolutionary processes leading to modified phenotypes in flightless species. This includes detailed studies on the thoracic skeletomuscular anatomy and function in more taxa, environmental factors potentially related to flightlessness, and also genetic and physiological regulatory networks related to structures linked with flight.

Major thoracic evolutionary changes and effects on the flight capacity

Limited morphological character systems, i.e. characters of specific body regions or life stages, are usually not sufficient to resolve the phylogeny of large and complex groups, especially when strong functional constraints are involved (see, e.g., Beutel et al. 2011, fig. 3). Apparently, this also applies to thoracic features of Lepidoptera, where even major evolutionary events (e.g., basal splits and the rise of Glossata) are not reflected by distinct modifications in this character system. Frequent homoplasy is an obvious problem, and also the serious lack of reliable data for crucial taxa, as pointed out in the "Introduction". Nevertheless, some major groups are well supported by evolutionary innovations in the thorax. These clades and major evolutionary trends and changes are treated in the following (see also list of apomorphies above in "Results").

The lepidopteran groundplan is mainly inferred from the thoracic characters of non-glossatan families, especially Micropterigidae, which is arguably the sistergroup of the remaining Lepidoptera and characterized by nearly equally sized pterothoracic segments and modest flying abilities (Kristensen and Skalski 1998, fig. 2.1; Kristensen 1998; Imada et al. 2011). A thoracic autapomorphy of Lepidoptera suggested in earlier studies is the presence of tergopleural apodemes in both pterothoracic segments (86.1) (tpa2/3: fig. 4d; Kristensen 1984, 2003; Kristensen and Skalski 1998; Friedrich and Beutel 2010). The mesothoracic tergopleural apodeme is the insertion point of M. mesopleura-praealaris (IItpm2) (Kristensen 2003). Several muscular apomorphies are suggested by the mapping analysis. However, these features are strongly affected by homoplasy or the polarity is ambiguous. As M. mesonoto-pleuralis posterior (IItpm6) (42.0) is also missing in Trichoptera (Maki 1938; Ivanov and Kozlov 1987), its absence apparently belongs to the amphiesmenopteran groundplan as suggested by Friedrich and Beutel (2010). M. mesopleura-sternalis (IIspm1) (45.1) is also present in Trichoptera (Maki 1938; Ivanov and Kozlov 1987) and most other groups of Holometabola (Friedrich and Beutel 2010). Consequently, its presence in Lepidoptera is very likely plesiomorphic. M. metanoto-sternalis (IIIdvm1)

(72.0) is absent in most non-ditrysian taxa but present in Hepialoidea and Adeloidea (Kozlov 2012). This muscle might be absent in the lepidopteran groundplan with several reversals, or parallel reductions occurred multiple times. A general trend is a gradual increase of the mesophragma index (Fig. 11c) from "lower" to "higher" lepidopteran groups (32.0) (Kristensen 2003; Nielsen and Kristensen 1996). The interpretation of a low mesophragma index as an apomorphy of Lepidoptera is likely an artifact. In any case, specific data for outgroup taxa are very limited. Obviously, more measurements for members of other holometabolan groups, especially Trichoptera, are required for a reliable evaluation.

Coelolepida (i.e., Glossata excluding Eriocraniidae) was supported as a clade in Regier et al. (2015) but not by recent analyses of transcriptomes, where Eriocranioidea were placed as the sistergroup of Neopseustoidea (Bazinet et al. 2016). Thoracic features tentatively suggest a monophyletic origin. Three potential apomorphies are the placement of the laterocervical posterior corner distinctly above the prokatepisternum or the pleurocoxal bridge (3.1) (Fig. 4c; plesiomorphy as in Fig. 12a), the articulation between the anteromedian pronotal part and the anterodorsal proepisternal corners (9.1) (Fig. 4c; plesiomorphy as in Fig. 12e) and the curling anterodorsal mesepimeral margin (21.1) (Fig. 4d; plesiomorphy as in Fig. 12c). Their absence in some coelolepidan families (e.g., Acanthopteroctetidae; Nielsen and Kristensen 1996) might be due to reversals. Alternatively, these apomorphies could characterize a less inclusive group than Coelolepida. Another plausible apomorphy of the coelolepidan wing base is the replacement of the metathoracic median plates by cubital plates (63.1) (cp3: Fig. 4e; plesiomorphy as in Fig. 12g). Like the mesophragma index, the ratio of the length of the postmedian mesonotal wing process + 1st axillary sclerite and the fore wing (22.1) (Fig. 4e; plesiomorphy as in Fig. 12b) also increases gradually with in evolution of Lepidoptera (Sharplin 1964b). Therefore, a ratio between 1/80 and 1/50 as an apomorphy of Coelolepida is possibly also an artificial result.

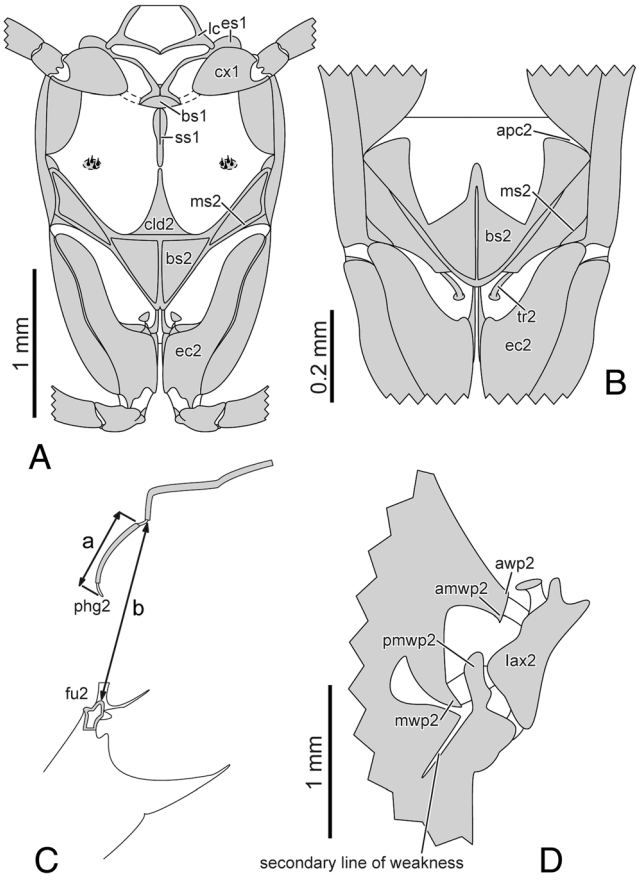


Fig. 11 Line drawing. **a** *Synempora andesae* (Neopseustidae), pro- and mesothorax in anteroventral view; **b** *Lophocorona pediasia* (Lophocoronidae), mesopleurosternum, anterior view; **c** mesophragma index (=**a**, **b**); **d** *Pieris brassicae* (Pieridae), mesothoracic first axillary sclerite from right fore wing. *Scale bars*, left 1 mm for (**a**); 0.2 mm for (**b**); right 1 mm for (**d**). **a**–**c** Redrawn from Nielsen and Kristensen (1996); **d** redrawn from Sharplin (1963a)

Heteroneura, which comprises ca. 99% of all lepidopteran species (Davis 1998), is characterized by an entire series of thoracic apomorphies, apparently reflecting a major evolutionary transition of this tagma. The main transformation compared with the lepidopteran groundplan is the enlargement of the mesothorax and shortening of the metathorax (Kristensen 2003). The absence of bending cuticle in ventral mesothoracic wing joint (25.1), which is present in Heliozelidae (Sharplin 1964b), is probably due to reversal. The mesothoracic tergopleural apodeme and subtegula loosely connect with each other (86.2), as an imtermediate state between completely separated and tightly fused. As the result of a gradual modification, this cannot be considered a precisely defined apomorphic state. A mesoclidium delimited from the posterior mesobasisternum (29.1) (cld2: Fig. 4b, c; plesiomorphy as in Fig. 11b) is also present in Neopseustidae (cld2: Fig. 11a; Nielsen and Kristensen 1996; Kristensen 2003) probably due to parallel evolution. The reduction of the metasubtegula (52.1) (plesiomorphy as in Fig. 12f) is linked with the presence of a free metabasalare (58.1) (bas3: Fig. 4d; plesiomorphy as in Fig. 12f) and the anterior shift of insertion of M. metapleura-praealaris (IIItpm2) from the metasubtegula to the metaprescutum or the pterothoracic intersegmental region (59.1) (plesiomorphy as in Fig. 12f). Further potential apomorphies of Heteroneura are several muscle losses, including dorsal longitudinal muscles II/IIIdlm3 (36.0, 71.0) and tergopleural muscles IIItpm3 and IIItpm10 (76.0, 79.0). However, these muscles are also present and highly variable in heteroneuran subgroups (Maki 1938; Nüesch 1953; Srivastava 1961, 1962; Ehrlich and Davidson 1961; Ehrlich and Ehrlich 1963; MacFarlane and Eaton 1973; Bharadwaj et al. 1974; Kozlov 2012). It is apparent that these characters are strongly affected by homoplasy. Nielsen and Kristensen (1996) suggested the presence of a proprecoxal bridge (10.1) (the sclerotized connection of proepisternum and

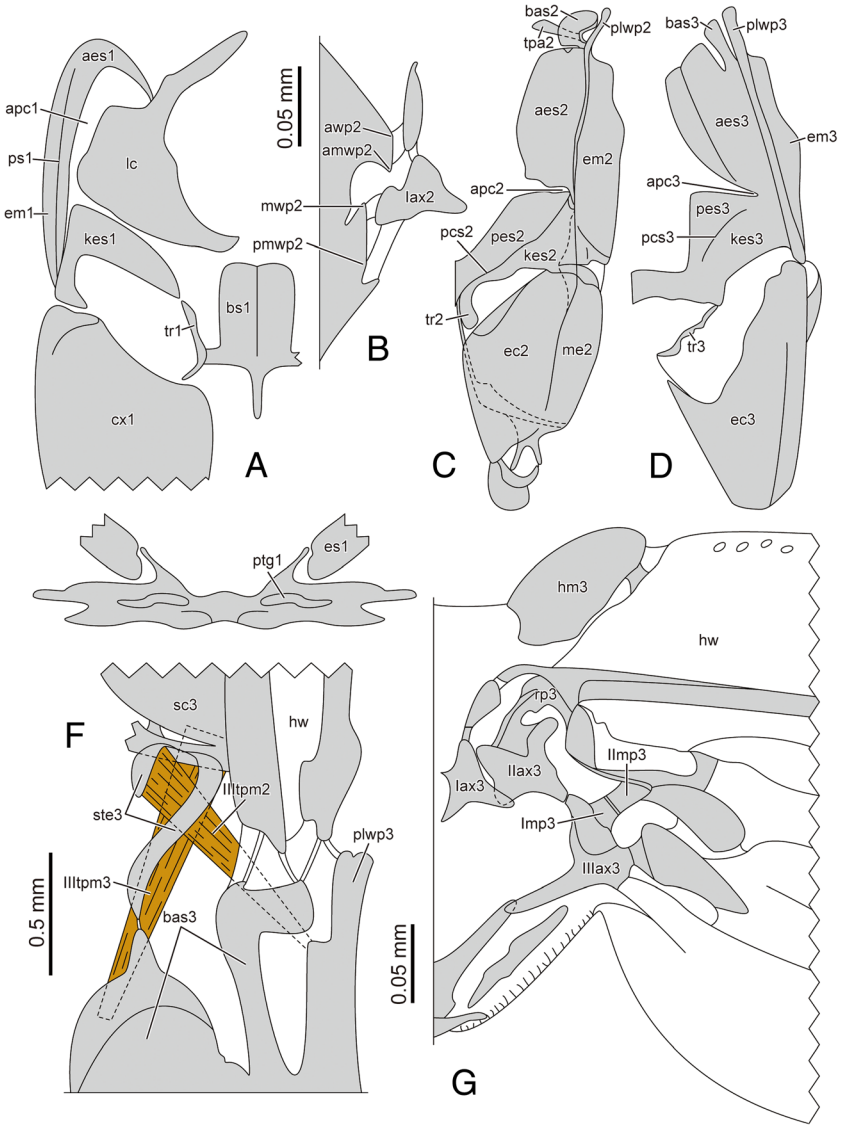


Fig. 12 *Micropteryx calthella* (Micropterigidae), line drawing, thoracic exoskeleton. **a** Ventral view of prothorax and neck region; **b** mesothoracic first axillary sclerite from right fore wing; **c** ventral view of mesopleuron; **d** ventral view of metapleuron; **e** dorsal view of pronotum; **f** basalar region of left hind wing base; and **g** dorsal view of right hind wing base. *Scale bars* upper 0.05 mm for (**b**); 0.5mm for (**f**); lower 0.05 mm for (**g**). **a**, c, **d** Redrawn from Matsuda (1970); **b** redrawn from Sharplin (1963a); **f**, **g** redrawn from Sharplin (1963b)

probasisternum: Fig. 4c; plesiomorphy as in Fig. 12a) as heteroneuran apomorphy. However, this feature is also present in Neopseustidae (Fig. 11a), whereas it is absent in the heteroneuran Opostegidae (Nielsen and Kristensen 1996; Kristensen 2003). This renders this apomorphy ambiguous and suggests a complicated evolutionary process. The presence of proprioceptive setae on the apical arm of laterocervicale (5.0) is an ambiguous character, as this feature also occurs in Hymenoptera and is quite variable in non-ditrysian groups (Nielsen and Kristensen 1996; Kristensen 2003).

Ditrysia, which are mainly based on a double opening of the female reproductive system, are moderately supported by thoracic features. Two thoracic apomorphies where suggested in previous studies (Kristensen 2003; Heikkilä et al. 2015), the V- or Y-shape mesoclidium (26.1) (Fig. 4b, c; plesiomorphy as in Fig. 11a) and the missing metatrochantin (62.0) (Fig. 4b, d; plesiomorphy as in Fig. 12d). The insertion of the M. metabasalare-trochantinalis (IIItpm2), which is already shifted anterad in the heteroneuran groundplan, is further moved forwards (59.2). The fusion of the mesosubtegula with the mesotergopleural apodeme is another potential apomorphy (86.3). However, a careful evaluation of this feature is required. In male *N. lefuarius*, for instance, the mesosubtegula is connected with the mesopleural wing process under the cuticular surface.

Within Ditrysia, only a few groups can be characterized by convincing thoracic apomorphies. The Y-shaped mesepimeral sulcus (20.1) is probably an autapomorphy of Sesiidae (Edwards et al. 1998). A mesophragma with dorsal processes (30.1) (Simonsen et al. 2012) and the loss of M. mesonotobasalaris (IItpm3) (39.0) are apomorphies of Papilionoidea. The

absence of IItpm3 is supported by all the previous anatomical studies of butterflies (Maki 1938; Srivastava 1962; Ehrlich and Davidson 1961; Ehrlich and Ehrlich 1963). The absence of this muscle in a species of Noctuidae (Bharadwaj et al. 1974) is apparently due to parallel loss. A complex apomorphy of Noctuoidea is the presence of a metathoracic tympanal organ (60.1) (Kitching and Rawlins 1998). The presence of sharp ventral processes of the tegula (13.1) is a potential apomorphy of Macroheterocera (in most Macroheterocera; Heikkilä et al. 2015). The blunt shape of this structure in some species of Bombycoidea (Heikkilä et al. 2015) is very likely a result of reversals.

A highly unusual feature occurring in Ditrysia is the secondary loss of the ability to fold back the wings. This seemingly ancestral "palaeopteran" condition has apparently evolved independently in members of Papilionoidea, Bombycoidea, and Geometridae (Sharplin 1963b), but not in any neopteran group outside of Lepidoptera. M. mes- and metepimero-axillaris tertius (II/IIItpm9), which initiate the folding process at the wing base (Sharplin 1964a), are retained in these species unable to fold back the wings (see Electronic Appendix 1), and are also present in the palaeopteran orders Ephemeroptera and Odonata (Willkommen and Hörnschemeyer 2007). It was pointed out by Brodsky (1994) that these muscles also play a role as flexors during the final stage of the wing movement. According to Sharplin (1964a), their contraction is only a trigger of the folding process, whereas the major mechanism is based on the configuration of the wing base sclerites. A large membranous area between the first and third axillary sclerites plays an important role, and also the twisting of a patch of bending cuticle at the distal end of the radial plate (Sharplin 1964a). The phylogenetic placement of the ditrysian taxa without wing folding clearly indicates that this condition has evolved several times independently. This is also supported by different structural features of the wing base in the three groups. In Papilionoidea, the second mesonotal line of weakness is present (Fig. 11d; Sharplin 1963a; Minet 1991), and the two mesothoracic median plates are reduced in size and disconnected from each other (Sharplin 1963a). The metathoracic median arm and cubital plates differ distinctly from the usual ditrysian arrangement in all three groups. However, the specific condition varies considerably among them, including not only reduction but also occasional enlargement (Sharplin 1963b). It is likely that different factors have played a role in the evolution of non-folding wings in those ditrysian groups. One possible reason is the presence of visual signals on the extensive scaled wings, which play a role in the reproduction of diurnal species (e.g. Beutel et al. 2014). Further investigations are required to elucidate the evolutionary background of this phenomenon. It should be noted in this context that the non-foldable wing base also differs strongly in the palaeopteran Ephemeroptera and Odonata (Willkommen and Hörnschemeyer 2007; Ninomya and Yoshizawa 2009). Consequently, it was suggested by the former authors that a "neopteran" wing base may be ancestral for the entire Pterygota and that a stiff wing base has evolved independently as an apomorphic condition in Ephemeroptera and Odonata, respectively.

Thoracic characters changes in the early evolution of Lepidoptera are mainly related to an enhancement of the flight capacity. A major transformation with the rise of Heteroneura is the enlargement of the mesothorax and shortening of the metathorax, with the former creating the main propulsive force in flight (anteromotorism) (Kristensen 2003). Other specific character transformations also can be seen in this context. The increasing size of the mesophragma is related to an enlargement of the principal wing depressor M. prophragma-mesophragmalis (IIdlm1) in lepidopteran evolution (Kristensen 2003; plesiomorphy as in Kozlov 1986a, Fig. 1). Another gradually modified character, the ratio of the length of the postmedian mesonotal wing process + 1st axillary sclerite and the fore wing, increases gradually from "lower" to "higher" lepidopteran grades (Figs. 4e and 12b; Sharplin 1964b). The two former structures as important components of the wing articulation play an important role in the earliest phase of wing depression (Brodsky 1994). Additionally, the mesothoracic 1st axillary sclerite is the insertion area of the direct flight muscle M. mesonoto-pleuralis posterior (IItpm4). The enlarged postmedian notal wing process and 1st axillary sclerite apparently result in a more powerful wing stroke. The curling anterodorsal mesepimeral margin (Fig. 4d) as the origin of the wing flexor M. mesepimero-axillaris tertius (IItpm9: Fig. 7f), probably leads to an increased efficiency of this muscle. Some characters are likely related to the shortened metathorax. This includes the reduction of the metathoracic median plates (Fig. 4e) and metasubtegula, the loss of three metathoracic muscles (IIIdlm3, IIItpm3, IIItpm10). The anterior shift of the insertion of M. metapleura-praealaris (IIItpm2) is probably linked with an improved coupling of the pterothoracic segments. Another evolutionary trend in Lepidoptera is to connect skeletal elements of the thorax. In the mesothorax, the tergopleural apodeme contacts with the subtegula, as a part of the wing base that apparently affects wing stroke movement. The mesoclidium connects with the prospinasternum with its anterior part, forming the prospinal apodeme (Figs. 4b and 7a). The articulation of the pronotum and proepisternum with the proprecoxal bridge establishes a sclerotized ring around the foramen occipitale (Fig. 4c). An increased stability of the thoracic segments may lead to a more effective control and protection during high speed or long distance flights.

The phylogenetic evaluation of thoracic features is impeded by different factors. A major problem is the ambiguity or inconsistency of presently available molecular (or morphology-based) phylogenetic hypotheses. Obviously, changes in the tree topology can strongly affect evolutionary interpretations. As shown by our analysis, the phylogenetic pattern and character evolution are also blurred by a high degree of homoplasy, with numerous possible

reversals or events of parallel evolution in the entire group. The character evaluation is also negatively affected by many gaps in the data set, which is due to the insufficient (or lacking) knowledge of the morphology of many groups, including phylogenetically crucial non-ditrysian taxa such as Heterobathmiidae, Lophocoronidae, and Acanthopterotetidae. The morphological investigations carried out during this study have also shown that the precise documentation of very small muscles is difficult (if possible at all) without microtome series or μ-CT data sets of very good quality. Therefore, it cannot be excluded that some muscular observations in earlier studies are incomplete or ambivalent. Another important factor is the outgroup selection. In our analysis, we used *Limnephilus marmoratus* as trichopteran outgroup, a taxon with well-documented thoracic features but deeply nested within the subgroup Integripalpia (Kjer et al. 2002). Apomorphies which may have independently evolved in this taxon may obscure the groundplan of Amphiesmenoptera and consequently also affect the basal splits within Lepidoptera.

Acknowledgements We are very grateful to Dr. Thomas Simonsen, Dr. Maria Heikkilä, Dr. Frank Friedrich, and an anonymous reviewer for their valuable comments, recommendations, and criticisms, which have enormously helped to improve this study. Their help with literature sources is also greatly appreciated. Great thanks are also due to Prof. Dr. Stanislav Gorb (Zoological Institute, Functional Morphology and Biomechanics, Kiel University) for providing μ-CT scans. This project is financially supported by the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 642241.

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3.2. Study II

Liu, S.-P., Wipfler, B. & Beutel, R.G. (2018)

The unique locomotor apparatus of whirligig beetles of the tribe Orectochilini (Gyrinidae, Coleoptera). *Journal of Zoological Systematics and Evolutionary Research*, 56(2): 196–208.

Abstract

This manuscript describes the external and internal thoracic structures of whirligig beetle *Orectochilus vilosus* (Coleoptera: Gyrinidae) in detail, with a series of traditional and advanced morphological techniques. Compared with the other groups of Coleoptera, *O. viosus* has extremely simplified pterothoracic skeletomusculatural structure with only 10 metathoracic muscles preserved. The metathoracic dorsal longitudinal muscles are absent in Gyrininae, and the limited other skeletomuscular elements take over more functions of flight. Meanwhile, the extensive and flat mesoventrite and the paddle-like pterothoracic legs are suitable for gliding on the water surface. The large dorsoventral muscle is responsible both as dominant wing levator and for powerful and rapid backstroke of hind legs.

Accepted: 4 September 2017

DOI: 10.1111/jzs.12195

ORIGINAL ARTICLE

WILEY JOURNAL OF ZOOLOGICAL SYSTEMATICS AND EVOLUTIONARY RESEARCH

The unique locomotor apparatus of whirligig beetles of the tribe Orectochilini (*Gyrinidae, Coleoptera*)

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Funding information
European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant, Grant/Award Number: 642241

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Abstract

Whirligig beetles, which are known for their rapid gliding on the water surface, have evolved a unique locomotor apparatus. External and internal thoracic structures of *Orectochilus villosus* (Orectochilini) are described in detail and documented with micro-computed tomography, computer-based 3D reconstructions, and scanning electronic microscopy (SEM). The results are compared with conditions found in other genera of *Gyrinidae* and other groups of Coleoptera. The focus is on structures linked with locomotion, especially on the unusual flight apparatus, which differs strongly from that of other beetles. As in the other Orectochilini, the prothorax of *Orectochilus* displays characters typical for *Gyrinidae*, with triangular procoxae and forelegs transformed into elongated, sexually dimorphic grasping devices. The musculature of this segment is similar to the pattern found in other Coleoptera. Similar to all other extant *Gyrinidae*, the mesothorax is characterized by an extensive and flat mesoventrite, suitable for gliding on the water surface. As in Heterogyrinae and the other Gyrininae, the pterothoracic legs are transformed into paddle-like structures, enabling the beetles to move with high speed on the surface film. The musculature of the mesothorax is reduced compared to other Coleoptera, but similar to what is found in the other Gyrininae. The metathoracic skeleton and musculature are simplified in Orectochilini compared to other Gyrininae and other groups of Coleoptera. In *O. villosus*, only 10 metathoracic muscles are preserved. 36 are present in an archostematan beetle, a condition probably close to the coleopteran ground plan. The metathoracic dorsal longitudinal bundles are absent in Gyrininae, muscles that play a role as indirect flight muscles in most other neopteran insects. The rest of the posteromotoric flight apparatus is distinctly modified, with a limited number of skeletomuscular elements taking over more functions. The large muscle M84 (IIIdvm7) M. noto–trochanteralis, for instance, functions as dominant wing levator, but is also responsible for the powerful and rapid backstroke of the hind legs. The presence of this muscle is a synapomorphy of Heterogyrinae and Gyrininae. The narrow metafurca in the latter group is likely linked to its large size. The elytra likely contribute to the control of the flight of the beetle, whereas they shield and inhibit the flight apparatus during swimming.

KEYWORDS
flight, *Gyrinidae*, locomotor apparatus, morphology, *Orectochilus*, thorax

1 | INTRODUCTION

Whirligig beetles or *Gyrinidae* are known for swimming rapidly in circles on the water surface, usually forming groups of several dozens or thousands of individuals (Bott, 1928; Larsén, 1966; Omer-Cooper, 1934). According to Nachtigall (1961), their swimming apparatus is the most efficient in the entire animal kingdom. The habitat with small arthropods caught in the surface film was referred to as a "world of the dead and the dying" (Omer-Cooper, 1934). The unusual life habits of whirligig beetles are linked with a series of autapomorphies, including the subdivided compound eyes and the antennae with fringes of setae on the pedicellus registering movements of the water surface (e.g., Larsén, 1966). The systematic position of the family within the suborder Adephaga is not fully settled (e.g., McKenna et al., 2015). However, a sister group relationship with all other adephagan families appears most likely, supported by morphological characters (Beutel & Roughley, 1988; Beutel et al., 2013) and also by a recent molecular study (Baca, Alana, Gustafson, & Short, 2017).

A comprehensive morphological study on the locomotor organs of Gyrinidae was published by Larsén (1966), together with the thoracic morphology of 54 species in the suborders Adephaga and Polyphaga. However, the work of Larsén (1966) was exclusively based on dissections and focused on the species *Gyrinus marinus* Gyllenhal, 1808 (Gyrinini). At that time, the gyrinid key taxon *Spanglerogyrus albiventris* Folkerts, 1979, the sister group of all the remaining genera, was unknown. Besides this, no information on the thoracic morphology of the "ancestral" suborder Archostemata was available (Baehr, 1975; Friedrich, Farrell, & Beutel, 2009).

In this study, we focus on the tribe Orectochilini, which includes the genera *Gyretes*, *Orectogyrus*, *Orectochilus* and *Patrus* (formerly a subgenus of *Orectochilus*; Miller & Bergsten, 2012). It is the subgroup with the most advanced features in this family according to phylogenetic analyses of Gyrinidae (Beutel, 1989a,b, 1990; Larsén, 1966; Miller & Bergsten, 2012). It cannot be excluded that some flightless species occur in Orectochilini (Larsén, 1954). However, there are records indicating that members of different genera of this tribe have retained their flight capacity (Brinck, 1984; Larsén, 1966; Ochs, 1966), including the one in the focus of this study, *Orectochilus villosus* (Müller, 1776). The thoracic morphology of this species is described in detail and documented using μ-CT data, computer-based 3D reconstructions and scanning electronic microscopy (SEM). The locomotor organs are discussed from evolutionary and functional perspectives, especially the flight apparatus, which is characterized by a distinctly modified skeletomuscular apparatus compared to beetles of other families or representatives of other groups of Neoptera (Brodsky, 1994; Larsén, 1966).

2 | MATERIALS AND METHODS

2.1 | Materials

Orectochilus villosus (Müller, 1776), fixed in FAE (formaldehyde-acetic acid-ethanol) and stored in ethanol, collected in the Saale river, 8 km south of Jena (Thuringia, Germany).

Heterogyrus milloti Legros, 1953 (Heterogyrinae; Miller & Bergsten, 2012), fixed and stored in 97% ethanol, collected at Fianarantsoa, small stream ~8 km W Ranomafana, Ranomafana NP, Madagascar, 21° 14.992′ S 47° 24.332′ E, 2 November 2014, Miller, Gustafson and Bergsten.

The specimens presently in the research are deposited in the Phyletisches Museum (Institut für Spezielle Zoologie und Evolutionsbiologie, FSU Jena). Additional morphological data were extracted from the literature (Beutel, 1989a,b, 1990; Friedrich et al., 2009; Larsén, 1954, 1966).

2.2 | Synchrotron radiation-based micro-computed tomography (SR-μCT)

One specimen was dehydrated in an ethanol series and dried at the critical point (EmiTech K850 Critical Point Dryer). It was scanned in a small Eppendorf tube at Beamline BW2 of German Electron Synchrotron Facility (DESY, Hamburg) using a stable low photon energy beam (8 keV) and absorption contract.

2.3 | Computer-based 3D reconstruction

Based on the μCT image stack, the thoracic segments of *O. villosus* were reconstructed three-dimensionally using FEI Amira 6.0. Segmented structures were exported as stacks of tiff files into Volume Graphics VGStudiomax 2.0, which was used for volume rendering.

2.4 | Scanning electronic microscopy

For the examination of external skeletal structures, a dried specimen was coated with gold (EmiTech K500 sputter coater). Micrographs were taken with Philips XL 30 ESEM and ResAlta Scandium software.

2.5 | Microscopic photography

To document the coloration and general body shape, the specimens were photographed with a Keyence VHX-2000.

2.6 | Line drawings

Exposed body parts of the specimen were drawn with full lines, structures below other sclerites with dotted lines. In Figure 3, wings and legs were omitted, except basal elements. In Figure 6, elytra are omitted. The figures were drawn with a pencil based on microscopic observations (with a camera lucida) or on 3D reconstructions, scanned, and finally completed with Adobe Illustrator CC.

2.7 | Measurements

Measurements were taken from digital photographs, SEM micrographs, line drawings and 3D reconstruction according to respective scale bars. The original scale bars can be accurate to 0.001 mm.

Considering the calculation error, we kept the accuracy to 0.01 mm. We consistently chose the longest portion in each dimension for measurement (e.g. ventrite of the mesothorax).

2.8 | Terminology

The terminology for the thoracic skeleton was adopted from Friedrich et al. (2009) and Larsén (1954, 1966). Muscle names of both Larsén (1966) and Friedrich and Beutel (2008) are used for easy comparison with other coleopteran taxa. Both studies were also used in the context of functional interpretations of thoracic structures. Basisubcostale (bsc) as a significant structure during wing stroke is adopted from Brodsky (1994).

2.9 | Abbreviations

1/2/3ax—first/second/third axillary sclerite; abd–abdomen; aest2/3—mes-/metanepisternum; alc—alacrista; anp—anterior notal process; ba3—metabasalare; bsc—basisubcostale; cx1/2/3—pro-/meso-/metacoxa;
dis—discrimen; el—elytron; elap—articulatory process of elytron; ep2/3—mes/metepimeron; epl1—proepipleuron; fem1/2/3—pro-/meso-/metafemur; fu1/2/3—pro-/meso-/metafurca; hw—hind wing; fup—profurcal process; mnp—median notal process; nt1—pronotum; ph1/2—pro-/mesophragma; pl1—propleuron; pls2/3—meso-/metapleural suture; pn3—metapostnotum; pnp—posterior notal process; prsc—prealar sclerite; pwp3—metathoracic pleural wing process; sa3—metasubalare; sc2/3—meso-/metascutum; scl2/3—meso-/metascutellum; scsh—mesoscutellar shield; se—spatulate setae; spi2—mesothoracic spiracle; tar1/2/3—pro-/meso-/metatarsus; tib1/2/3—pro-/meso-/metatibia; tr1/2/3—pro-/meso-/metatrochanter; v1/2/3—pro-/meso-/metaventrite.

3 | RESULTS

3.1 | Thoracic morphology of *Orectochilus villosus*

3.1.1 | General appearance

Body length: 9.65 mm, width: 3.83 mm, height: 2.86 mm (length: width: height ratio = 3.4: 1.3: 1.0). The thorax appears compact, stream-lined, and laterally compressed, with a flat ventral surface and a convex dorsal side. The pronoto-elytral angle is indistinct. The pronotal and elytral surfaces (Figure 1a) bear a dense vestiture of fine setae.

3.1.2 | Prothorax

Length: 1.87 mm (pronotum), width: 3.21 mm (pronotum), height: 2.16 mm (length: width: height ratio = 1.0: 1.7: 1.2). Cervical sclerites are missing. The head and prothorax are connected by a cervical membrane, which is not visible externally. A dense pubescence is present on the entire pronotal surface. The anterior and posterior margins of the convex pronotum (nt1: Figures 1a,c; 3c; 5a) are not distinctly extended. The anterior pronotal area covers the occipital region of the head and appears almost merged with it. The anterior pronotal margin is very finely serrated. The proepipleuron (epl1: Figures 1b; 3c; 5a) is narrow as in the other Orectochilini (Larsén, 1966). The propleuron (pl1: Figures 1b; 3c; 5a) is delimited by a suture extending obliquely from the anteroventral margin of the epipleuron to the prothoracic pleurocoxal joint. The internalized cryptopleura are extended mesad and fused with the pronotum, only leaving a narrow tergal region for muscle attachment. This is a character shared in Orectochilini and Enhydrini (Larsén, 1966; Baehr, 1979). The posterior wall (Figure 3c) of the prothorax is formed by the posterior pronotal area, the posterolateral epipleural area, and the transverse concave ventral propleural lobe. The posterior pronotum (nt1: Figure 3c) is narrow in *O. villosus*, but even narrower in the other Orectochilini (Larsén, 1966). The line separating the epipleural and propleural elements of the posterior wall is indistinct, probably an autapomorphy of Orectochilini (Larsén, 1966). A projecting process bears a tuft of spatulate setae (se: Figure 3c), which form the proprioceptive sense organ located ventrolaterally on the posterior segmental wall, as in other Orectochilini (Larsén, 1966). The process is short and rounded in *O. villosus* and *Gyretes*, but blunt in *Orectogyrus* (Larsén, 1966). The proventrite (Figures 2a; 3c) is bulging medially as in *Orectogyrus* (Larsén, 1966), gradually shortened paramedially and laterally connected with the propleura. Medially it is divided by a distinct median ridge, and another ridge is present along its posterior margin. The prosternal process is reduced, not reaching between the procoxae. The profurca (fu1: Figures 3c; 4c) bears a long posterior arm, which is bent laterad and reaches deeply into the mesothoracic lumen as in other Orectochilini and Enhydrini (Larsén, 1966; Baehr, 1979; Beutel, 1989b). Anteriorly it bears a vertically oriented profurcal process (fup: Figure 3c).

The forelegs (Figures 1b; 2a) are modified as elongate grasping devices. The procoxa (cx1: Figures 2a; 4a; 3c; 5a) is triangular and only slightly protruding. It lacks a ventral condyle articulating with the prosternal process. The triangular protrochanter (tr1: Figures 2a; 3c; 5a), equipped with a group of setae ventrally, connects the procoxa with the elongate profemur (fem1: Figure 1b; 2a) (length: 1.83 mm, width: 0.46 mm; length: width ratio = 4.0: 1.0). The anterior profemoral margin bears a row of spines. The elongate protibia (tib1: Figures 1b; 2a; length: 1.47 mm, width: 0.26 mm; length: width = 5.7: 1.0), basally narrow and distally widening, bears a mesal comb of spines. Apical tibial spurs are missing. The sexually dimorphic laterally compressed protarsus (tar1: Figures 1b; 2a) is composed of five tightly connected tarsomeres. The tarsal segments in males are equipped with a brush-like dense vestiture of adhesive hairs with apical suckers. The distal tarsomere bears a pair of curved claws.

Musculature (Figures 4; 5; see Table 1 for an overview of all muscles; abbreviations from Friedrich and Beutel (2008) in brackets): M1 (Idlm2) M. pronoti primum: O (= origin): anteromedian area of pronotum; I (= insertion): dorsolateral area of occipitale. M2 (Idlm1) M. pronoti secundus, bent upwards: O: first phragma; I: dorsolateral

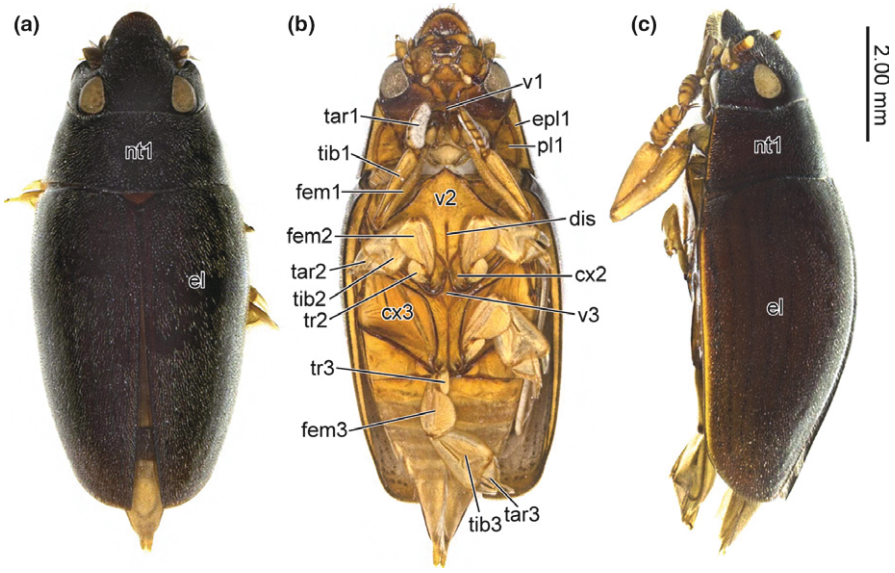

FIGURE 1 *Orectochilus villosus*, digital photographs, male habitus. (a) dorsal view; (b) ventral view; (c) lateral view

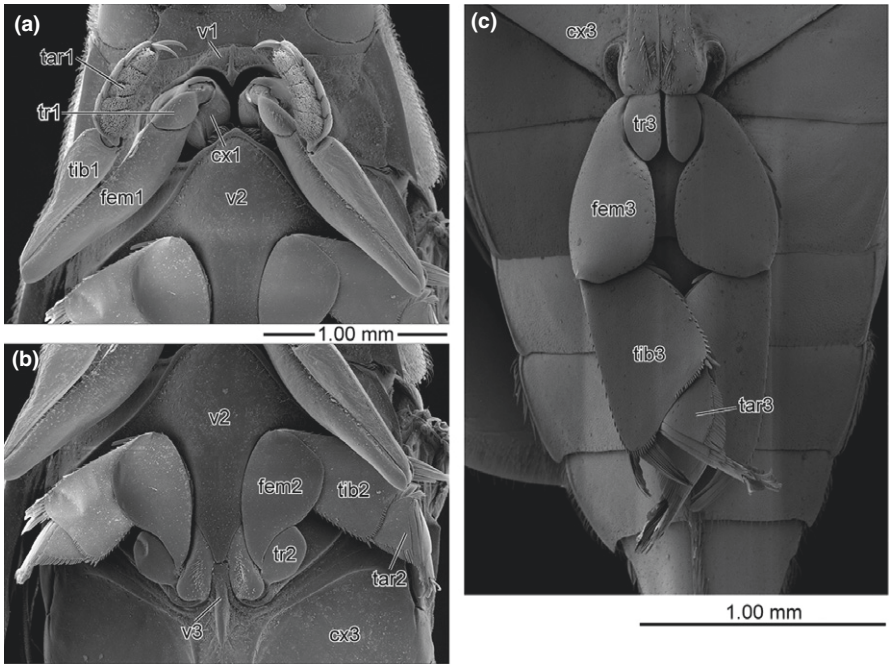

FIGURE 2 *Orectochilus villosus*, SEM micrographs, legs (male). (a) foreleg; (b) midleg; (c) hind leg

area of occipitale. M3 (Idlm3) M. pronoti tertius, bent upwards: O: first phragma; I: anterior pronotal margin. M5 (Ivlm3) M. prosterni primus: O: basal area of profurca; I: ventrolateral area of occipitale. M6 (Ivlm1) M. prosterni secundus: O: profurcal process; I: ventral cervical membrane. M7 (Idvm6) M. dorsoventralis primus, broader on pronotum, narrowing toward occipitale: O: central area of pronotum; I: ventrolateral area of occipitale. M10 (Idvm2/3) M. dorsoventralis quartus: O: anterior margin of prosternum; I: dorsolateral area of occipitale. M11 (Idvm10) M. dorsoventralis quintus, triangular muscle, narrower on mesoscutum; broadening toward profurca: O: dorsal area of profurca; I: anterolateral area of mesoscutum. M13 (Itpm6) M. pronoto–mesepisternalis, broader on pronotum, narrowing toward mesanepisternum: O: central area of pronotum; I: intersegmental membrane anterior to mesanepisternum. M14 (Idvm13) M. noto–trochantinalis, broader on pronotum, narrowing toward procoxa: O: anterolateral area of pronotum; I: lateral procoxal rim. M15 (Idvm16/17) M. noto–coxalis, broader on pronotum, narrowing toward procoxa: O: lateral area of pronotum; I: posterior procoxal rim. M16 (Ipcm4) M. epimero–coxalis, broader on propleuron, narrowing toward procoxa: O: anterodorsal area of propleuron; I: anterolateral procoxal rim. M20 (Ipcm8) M. pleura–trochanteralis, broader on prothoracic posterior wall, narrowing toward trochanter: O: prothoracic posterior wall; I: protrochanter. M21 M. pleura–trochanteralis medialis, wide area of origin on procoxa, converging toward protrochanter: O: procoxal mesal wall; I: protrochanter. M22 M. coxa–trochanteralis lateralis, at least two bundles, wide area of origin on procoxa, converging toward protrochanter: O: lateral procoxal wall; I: protrochanter.

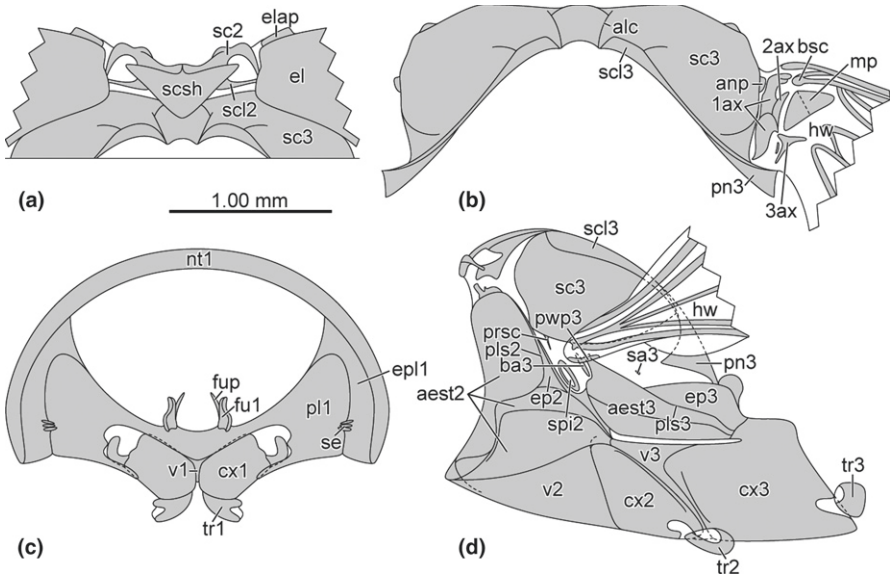

FIGURE 3 *Orectochilus villosus*, line drawing, thoracic skeleton. (a) dorsal view of mesothorax; (b) dorsal view of metathorax; (c) posterior view of prothorax; (d) lateral view of pterothorax

The prothoracic muscles, especially those that attach to the cervical region and legs, are similar to those of the other examined species of Gyrinidae or other groups of Coleoptera (Baehr, 1975; Beutel, 1989b; Friedrich & Beutel, 2006; Friedrich et al., 2009; Larsén, 1966). The modifications compared with the general coleopteran muscular pattern reported by Larsén (1966: table II) and Beutel and Haas (2000: appendix IV) are the following: M4 (Idlm5) M. pronoti quartus, M8 (Idvm8) M. dorsoventralis secundus, M9 (Idvm5) M. dorsoventralis tertius, M12 (Itpm3?) M. pronoto–pleuralis, M17 (Ipcm8) M. epimero–coxalis, M18 (Iscm1) M. sterno–coxalis and M19 (Iscm2) M. furca–coxalis are absent. The origin of M1 (Idlm2) M. pronoti primum is shifted anterad. The origin of M7 (Idvm6) M. dorsoventralis primus is shifted mesad. The original area of one subcomponent of M15 (Idvm16/17) M. noto–coxalis is transverse and shifted to the posterolateral region of the pronotum.

3.1.3 | Mesothorax

Length 1.62 mm (mesoventrite+mesocoxa), width 3.48 mm (mesoventrite), height 1.76 mm (from mesoscutellar shield to mesoventrite, without elytra) (length: width: height ratio = 1.0: 2.1: 1.1). A flat median concavity is present at the anterior margin of the mesoscutum (sc2: Figure 3a), and both sides of the sclerite are protruding anteriorly. Paired, distally rounded processes extending posteroventrad from the anterior concavity form the prophragma (ph1: Figure 4c) in *O. villosus*. It is a single and apically truncated structure in *Orectogyrus* and also a single median lobe in a few species of *Gyretes*, whereas it is bilobed in the other Gyrinidae (Hatch, 1926; Larsén, 1966). The narrow sclerotized lateral edge of the mesoscutum lacks recognizable notal processes. An axillary ligament connects it with the articulatory process of the elytron (elap: Figure 3a). The area between the mesoscutum and the posterior mesoscutellum is unsclerotized, nearly membranous. The mesoscutellum (scl2: Figure 3a) bears a sclerotized triangular mesoscutellar shield (scsh: Figure 3a) on its middle region, and its lateral parts are very narrow. The mesopleuron is triangular. The mesanepisternum (aest2: Figure 3d) bears a small process on its dorsal margin, arguably a reduced mesobasalare. As in other Orectochilini, the opening between the elytron and laterally expanded dorsal margin of the mesanepisternum is narrow in *O. villosus* (Larsén, 1966), an autapomorphy of the tribe (Beutel, 1990). The ventral mesanepisternal margin broadly connects with the dorsolateral margin of the mesoventrite (v2: Figures 1b; 2a,b; 3d) and meets the lateral mesocoxal edge posteriorly. This ventral margin forms an excavation for the profemora in the resting position. Posteromedially, the mesanepisternum is adjacent to the narrow triangular mesepimeron (ep2: Figure 3d), both separated by the mesopleural suture (pls2: Figure 3d). The mesokatepisternum is completely fused with the main part of the mesoventrite, without a transverse ridge. Anteriorly the mesoventrite forms a triangular process that bears two separate groups of setae. Posteromedially a discrimen (dis: Figure 4a) is present protruding dorsad into the mesothoracic lumen. The short but well-developed mesofurca (fu2: Figure 4b) originates dorsally on the discrimen. It bears a large apical disk for muscle attachment.

The mesocoxae are approximately triangular (cx2: Figures 1b; 3d; 4a; 5a), medially adjacent and diverging anterolaterally. The anterior edge is immovably attached to the posterior margin of the mesoventrite. This is also the case in *Orectogyrus*, whereas a certain movability is retained in *Gyretes* (Larsén, 1966). The median line separating the round median mesocoxal lamellae is anteriorly continuous with the discrimen. Anterolaterally, an extensive triangular apodeme is present for muscle attachment. Dorsally, the large trochanteral tendon for muscle attachment expands to the region of the mesoventrite and the mesanepisternum. Compared with generalized coleopteran walking legs, the distal parts of the middle legs are strongly modified. The mesotrochanter (tr2: Figures 1b; 2b; 3d; 4a; 5a) connects the mesocoxa with the shortened, flattened, and roughly triangular mesofemur (fem2: Figures 1b; 2b; 5a; length: 0.77 mm, width: 0.45 mm; length: width ratio = 1.7: 1.0). The

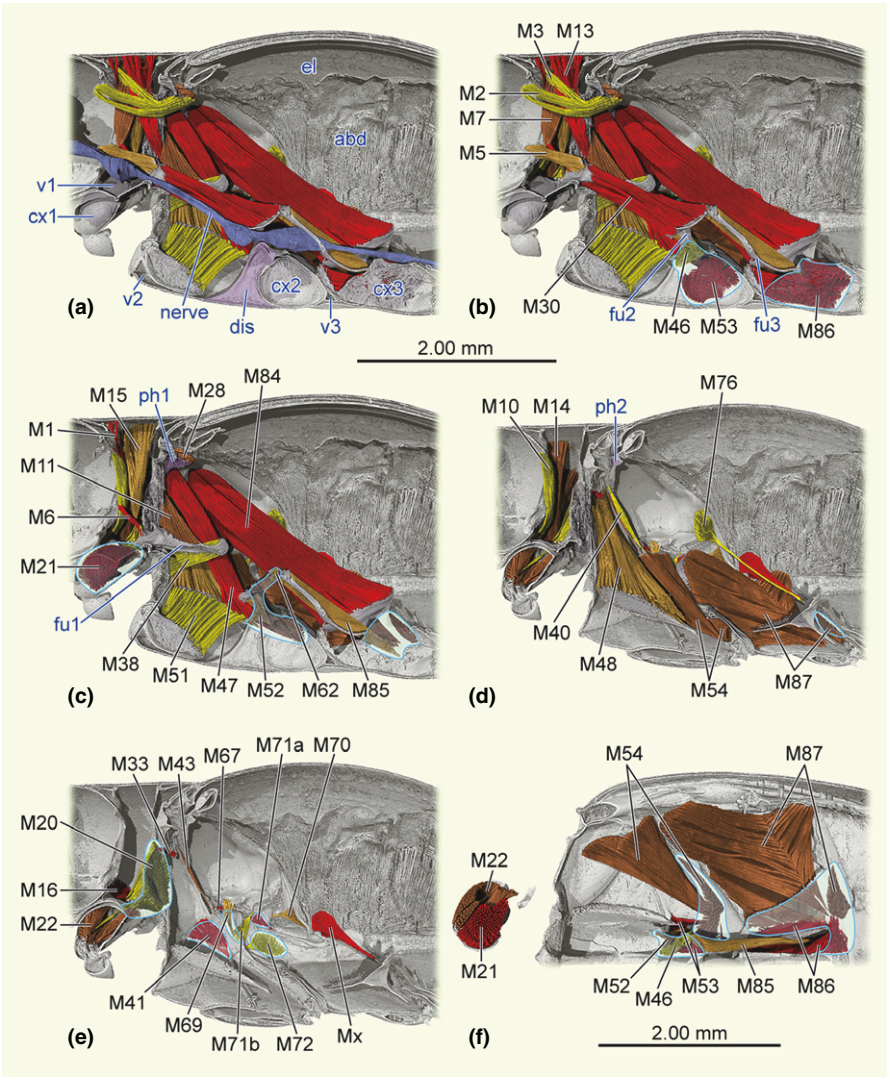

FIGURE 4 *Orectochilus villosus*, 3D reconstruction, thoracic endoskeleton and muscles. Skeletal structures in blue lines rendered transparent to show muscles behind them. Skeletal structures labeled in blue, muscles in black. (a–e) lateral view, dis and ph1/2 in pink; F: dorsal view, coxal muscles. Scale bars: upper 2.00 mm for (a–e) lower 2.00 mm for (f)

mesofemur broadly connects with the similarly broadened and flattened mesotibia (tib2: Figures 1b; 2b; 5a; length: 0.75 mm, width: 0.52 mm; length: width ratio = 1.4: 1.0), which bears swimming lamellae anterodistally and a row of spines posteriorly. The mesotarsus (tar2: Figures 1b; 2b) inserts onto the apical mesotibial margin. Its posterior margin is also equipped with swimming lamellae. The internal walls of the mesotibiae and the proximal tarsomere are connected by the cuticular columnae, as in the other Orectochilini and Enhydrini (Beutel, 1990; Larsén, 1966). The five tarsomeres are flattened, together forming a fan-shaped structure. The distal margin of the tarsomere 4 is bent dorsad, thus forming a shovel-like structure with the shape of the proximal 3 tarsomeres, similar to the condition found in other Orectochilini and some Enhydrini (Beutel, 1990; Larsén, 1966). Tarsomere 5 is proximomesally connected with tarsomere 4 and bears a pair of curved claws apically.

The elytra (el: Figures 1a,c; 5a) are posteriorly truncated and bear a dense vestiture of short and thin setae, similar to the pronotal vestiture. The pubescent dorsal side of the body is a diagnostic character of *Spanglerogyrus*, *Heterogyrus*, and Orectochilini (Folkerts, 1979; Larsén, 1966; Miller & Bergsten, 2012). Elytral striae are absent. The glossula is present, as in the other Orectochilini and Enhydrini (Beutel, 1990; Larsén, 1966).

Musculature (Figures 4; 5; Table 1): M28 (IIdlm1) M. mesonoti primus: O: first phragma; I: second phragma. M30 (Ivlm7) M. mesosterni secundus: O: basal area of profurca; I: mesofurcal arm. M33 (IItpm2) M. noto–pleuralis: O: process of mesopleural ridge; I: dorsolateral area of intersegmental membrane between pro- and mesothorax. M38 (Ispm5) M. profurca–mesepisternalis: O: profurcal arm; I: anterior margin of mesanepisternum. M40 (IIdvm4/5) M. noto–coxalis, slightly broader medially: O: lateral area of mesoscutum; I: posterolateral mesocoxal rim. M41 (IIpcm4) M. episterno–coxalis, broader on mesanepisternum, narrowing toward mesocoxa: O: central area of mesanepisternum; I: anterolateral mesocoxal rim. M43 (IIdvm6) M. coxa–subalaris, slightly broader medially: O: membranous area between mesoscutum and mesanepisternum; I: posterolateral mesocoxal rim. M46 (IIscm2) M. furca–coxalis posterior, broader on mesofurca, narrowing toward mesocoxa: O: basal area of mesofurca; I: posterior mesocoxal rim. M47 (IIdvm7) M. noto–trochanteralis: O: mesoscutum; I: mesotrochanter. M48 (IIpcm6) M. episterno–trochanteralis, triangular, narrower on mesanepisternum, broadening

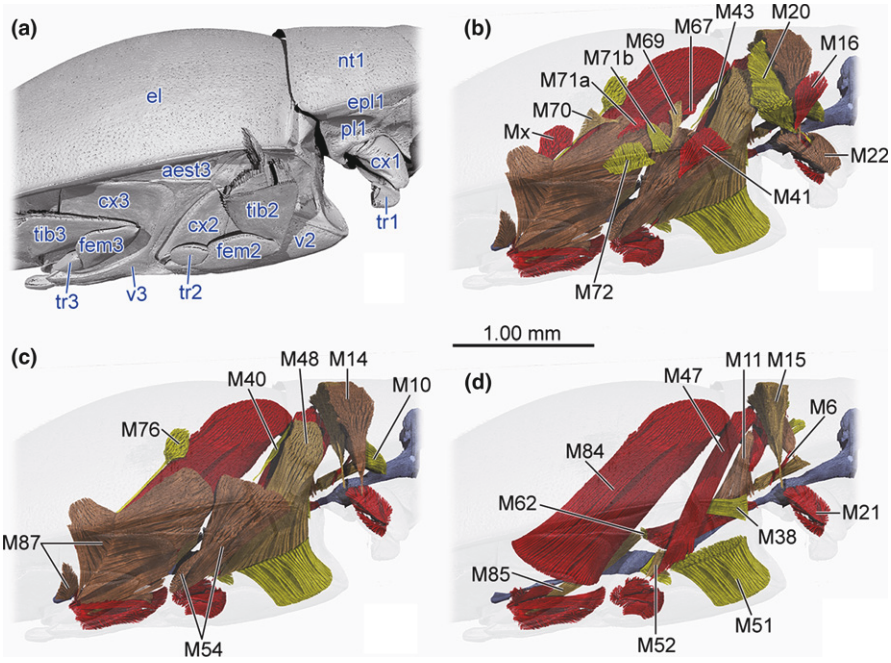

FIGURE 5 *Orectochilus villosus*, 3D reconstruction, thoracic skeleton and muscles. Skeletal structures labeled in blue, muscles in black. (a) lateral view, exoskeleton; (b–d) lateral view, skeleton transparent

toward mesotrochanter: O: dorsal area of mesanepisternum; I: mesotrochanteral tendon. M51 (?) M. sterno–trochanteralis: O: invaginated area of mesoventrite; I: mesotrochanteral tendon. M52 (IIscm6) M. furca–trochanteralis, broader on mesofurca, narrowing toward mesocoxa: O: anteroventral area of mesofurcal arm; I: mesotrochanter. M53 M. coxa–trochanteralis medialis, several bundles, distributed over wide area of mesocoxa, converging toward mesotrochanter: O: mesocoxal anterior rim and median wall; I: mesotrochanter. M54 M. coxa–trochanteralis lateralis, two bundles, the anterior one larger, the posterior one shorter: O: lateral mesocoxal wall; I: mesotrochanter.

The muscular apparatus of the mesothorax comprises only 12 muscles (excl. M53 and M54 as intrinsic mesocoxal muscles), compared to 25 in a more plesiomorphic pattern in other groups of Adephaga and in Polyphaga (Larsén, 1966; Beutel & Haas, 2000), and 33 in the archostematan *Tetraphalerus bruchi* Heller, 1913 (Friedrich et al., 2009). As stated in Larsén (1966), M29 (IIdlm2) M. mesonoti secundus, M31 (Ivlm9) M. mesosterni secundus, M32 (IIdvm8) M. dorsoventralis, M34 (IItpm10?) M. noto–epimeralis, M35 (IItpm10) M. epimero–subalaris, M36 (IItpm7 & 9) M. pleura–alaris, M37 (IIspm2) M. furca–pleuralis, M39 (IIdvm2) M. noto–trochantinalis, M42 (IIpcm3) M. episterno–coxalis, M44 (IIscm1) M. furca–coxalis anterior, M45 (IIscm4) M. furca–coxalis lateralis, M49 (?) M. epimero–trochanteralis, and M50 (IIpcm5) M. trochantero–basalaris are absent.

3.1.4 | Metathorax

Length: 0.98 mm (metacoxa), width: 3.28 mm (paired metacoxae), height: 1.87 mm (from central metascutal area to level of ventral mesocoxal surface) (without hind wings) (length: width: height = 1.0: 3.3: 1.9. The propulsive force during flight is only created by the metathorax as in the other beetles and in Strepsiptera (posteromotorism; e.g., Friedrich & Beutel 2010). The dorsal metathoracic parts are large compared with the corresponding mesothoracic elements. The median portion of the metascutum (sc3: Figure 3a,b,d) is short and lacks a membranous area, but is widened laterally, as in other Orectochilini and Enhydrini (Beutel, 1990; Hatch, 1926; Larsén, 1966). The paired processes of the anterior metascutal margin bend posteroventrad into the thoracic lumen, thus forming the mesophragma (ph2: Figure 4d). Anterolaterally the metascutum bears a very small sclerotized process, the metaprealar sclerite (prsc: Figure 3d), similar to a homologous structure in *Orectogyrus* (Larsén, 1966). The alacristae (alc: Figure 3b) are short (length: 0.24 mm) but distinctly developed, with a sharp posterior edge. A dense field of microtrichia is present on a transverse posterior metascutal concavity. The metascutellum (scl3: Figure 3b,d) attaches to the posterior metascutal margin. Posterolaterally the postnotum (pn3: Figure 3b,d) is inflected below the mesoscutum. Its lateral part forms a triangle, which is distinctly widening laterally. No metaphragma is developed. The oblique metapleural suture (pls3: Figure 3d) divides the metapleuron into the anterior metanepisternum (aest3: Figure 3d) and posterior metepimeron (ep3: Figure 3d). The posterior edge of the narrow metabasalare (ba3: Figure 3d) runs parallel to the metathoracic pleural wing process (pwp3: Figure 3d) and comes close to the dorsal area of the metanepisternum ventrally. The metabasalare of other species in *Orectochilus* and Orectochilini is solidly attached to the metanepisternum (Larsén, 1966). The metasubalare (sa3: Figure 3d) is very small (length: 0.02 mm, height: 0.02 mm) and embedded in the membranous area above the sclerotized metapleuron. The metanepisternum is narrower than in *Orectogyrus* and *Gyretes* (Larsén, 1966). The metepimeron connects with the lateral metapostnotal edge posterodorsally, and with a semicircular disk-like element formed by the first abdominal pleuron.

TABLE 1 The thoracic musculature of Orectochilini

Larsén (1954)	Friedrich and Beutel (2008)	*Orectochilus villosus*	*Orectogyrus ornaticollis*	*Gyetes zimmermanni*
Prothorax				
M1	Idlm2	+	+	-
M2	Idlm1	+	+	+
M3	Idlm3	+	+	+
M4	Idlm5	-	-	-
M5	Ivlm3	+	+	+
M6	Ivlm1	+	+	+
M7	Idvm6	+	+	+
M8	Idvm8	-	-	-
M9	Idvm5	-	-	-
M10	Idvm2/3	+	+	+
M11	Idvm10	+	+	+
M12	Itpm3?	-	-	-
M13	Itpm6	+	+	+
M14	Idvm13	+	+	+
M15	Idvm16/17	+	+	+
M16	Ipcm4	+	+	+
M17	Ipcm8	-	-	-
M18	Iscm1	-	-	-
M19	Iscm2	-	-	-
M20	Ipcm8	+	+	+
M21	/	+	/	/
M22	/	+	/	/
Mesothorax				
M28	IIdlm1	+	+	+
M29	IIdlm2	-	-	-
M30	Ivlm7	+	+	+
M31	Ivlm9	-	+	+
M32	IIdvm8	-	-	-
M33	IItpm2	+	+	-
M34?/35	IItpm10	-	-	-
M36a	IItpm9	-	-	-
M36b	IItpm7	-	-	-
M37	IIspm2	-	-	-
M38	Ispm5	+	+	+
M39	IIdvm2	-	-	-
M40	IIdvm4/5	+	-	-
M41	IIpcm4	+	-	+
M42	IIpcm3	-	-	-
M43	IIdvm6	+	-	-
M44	IIscm1	-	-	-
M45	IIscm4	-	-	-
M46	IIscm2	+	-	+
M47	IIdvm7	+	+	+
M48	IIpcm6	+	+	+
M49	/	-	-	-
M50	IIpcm5	-	-	-
M51	?	+	+	+
M52	IIscm6	+	+	+
M53	/	+	/	/
M54	/	+	/	/
Metathorax				
M60	IIIdlm1	-	-	-
M61	IIIdlm2	-	-	-
M62	IIvlm3	+	+	+
M63	IIvlm5	-	-	-
M64	IIIdvm1	-	-	-
M65	IIIdvm8	-	-	-
M66	IIIdvm8	-	-	-
M67	IIItpm2	+	+	+
M68	IIItpm6	-	-	-
M69	IIItpm3	+	-	-
M70	IIItpm10	+	+	+
M71a	IIItpm9	+	-	-
M71b	IIItpm7	+	-	-
M72	IIIppm1	+	+	+
M73	IIIspm1	-	-	-
M74	IIIdvm2	-	-	-
M75	IIIdvm4	-	-	-
M76	IIIdvm5	+	+	+
M77	IIIpcm4	-	-	-
M78	IIIpcm3	-	-	-
M79	IIIdvm6	-	-	-
M80	IIscm7?	-	-	-
M81	IIIscm1	-	-	-
M82	IIIscm4	-	-	-
M83	IIIscm2	-	-	-
M84	IIIdvm7	+	+	+
M85	IIIscm6	+	+	+
M86	/	+	/	/
M87	/	+	/	/
Mx	/	+	/	/

(Muscular nomenclatures from Larsén (1966) and Friedrich and Beutel (2008) are listed, respectively, in the first and second rows according to homology (Friedrich et al., 2009). Here, we revise M38 as homologous to Ispm5 instead of Ispm6, according to the origin and insertion of this muscle on the same body side. Muscle present is represented by "+" in green, absent with "-" in orange, uncertain with "?" or "/"in yellow).

The metaventrite (v3: Figures 1b; 2b; 3d; 4a; 5a) reaches its maximum length at midline, with narrow oblique lateral wings enclosed between the posterior margin of the mesocoxae and the anterior margin of the metacoxae. The metakatepisternum is fused

with the ventrite, without a trace of a transverse ridge. The discrimen is also lacking, and the metatrochantin is not visible externally. The metaventrite bears a narrow median process (width: 0.68 mm) fitting between the posteromedian mesocoxal edges. A visible cleft separates the dorsal metanepisternum from the ventral metaventrite and metacoxa. The metacoxae (cx3: Figures 1b; 2b,c; 3d; 4a; 5a) are greatly enlarged compared to most other beetles and rectangular, extending far anterolaterad as in other Orectochilini and Gyrinini (Hatch, 1926; Larsén, 1966). The oblique anterior margin is fused with the posterior margin of the metaventrite. An anterolateral extension reaches into the metathoracic lumen as a disk-like structure for muscle attachment. The fused median metacoxal lamellae are longer than those of the mesocoxae. The connection area of the paired metacoxae is visible as a median suture as in *Heterogyrus* and other Gyrininae (Larsén, 1966). The narrow metafurca (fu3: Figure 4b) with paired parallel arms extends anterodorsad from the metacoxal lamella and almost reaches the mesofurca anteriorly. The hind legs as a whole are larger than the middle legs. Their distal parts are similar, also forming shortened paddle-like structures for swimming (metafemur length: 0.98 mm, width: 0.72 mm; length: width ratio = 1.4: 1.0. metatibia length: 1.11 mm, width: 0.79 mm; length: width ratio = 1.4: 1.0). The internal walls of the metatibiae (tib3: Figures 1b; 2c; 5a) and the proximal tarsomere are connected by cuticular columnae (Larsén, 1966). Tarsomere 4 is similar to its mesothoracic equivalent, also forming a shovel-like structure with the proximal 3 tarsomeres. This structure is similar to the condition found in other Orectochilini and Enhydrini (Beutel, 1990; Larsén, 1966).

The sclerotized anterior notal process (anp: Figure 3b) is attached to the middle region of the lateral metascutal margin. The elongate first axillary sclerite (1ax: Figure 3b) is separated into two parts by a suture in its middle section, and its anteroproximal part is close to the basisubcostale (bsc: Figure 3b). The narrow second axillary sclerite (2ax: Figure 3b) is tightly adjacent to the distal portion of the mesal margin of the first axillary sclerite. The third axillary sclerite (3ax: Figure 3b) bears three processes and a small proximal sclerite close to the posterior part of the first axillary sclerite (length: 0.80 mm, width: 0.11 mm). The median plate (mp: Figure 3b) is weakly sclerotized and not distinctly delimited from the membranous area of the wing base. It is divided by a fold along its midline.

Musculature (Figures 4; 5; Table 1): M62 (IIvlm3) M. metasterni primus: O: posterior surface of mesofurcal arm; I: anteriorly on metafurcal arm. M67 (IIItpm2) M. pleura–praealaris: O: metapleural wing process; I: metaprealar sclerite. M69 (IIItpm3) M. noto–basalaris, elongated triangular: O: lateral margin of metascutum; I: metabasalare. M70 (IIItpm10) M. epimero–subalaris, triangular, broader on metapostnotum, narrowing toward metasubalare: O: posteroventral margin of metapostnotum; I: metasubalare. M71a (IIItpm9) M. pleura–alaris a, triangular, broader on metapleural ridge; narrowing toward third axillary sclerite: O: median area of metapleural ridge; I: third axillary sclerite. M71b (IIItpm7) M. pleura–alaris b, elongated triangular, broader on metanepisternum, narrowing toward third axillary sclerite: O: anterodorsal area of metanepisternum; I: third axillary sclerite. M72 (IIIppm1) M. sterno–episternalis: O: anterodorsal area of metanepisternum; I: dorsal area of metaventrite. M76 (IIIdvm5) M. noto–coxalis posterior, broad origin on metascutum, converging on a tendon inserted on metacoxa: O: posterolateral area of metascutum; I: posterolateral metacoxal rim. M84 (IIIdvm7) M. noto–trochanteralis: O: dorsal area of metascutum; I: large disk-shape apodeme of metatrochanter. M85 (IIIscm6) M. furca–trochanteralis: O: metafurcal arm; I: metatrochanter. M86 M. coxa–trochanteralis medialis, several bundles: O: anterior metacoxal rim and mesal wall; I: metatrochanter. M87 M. coxa–trochanteralis lateralis, two bundles, the anterior one stronger with fibers converging medially, the posterior one shorter: O: lateral metacoxal rim; I: metatrochanter. Mx, elongated conical, broader on first abdominal pleuron, narrowing toward metacoxa: O: semicircular disk of first abdominal pleuron; I: posterolateral metacoxal rim.

The metathoracic muscular apparatus comprises only 10 muscles (excl. M86 and M87 as intrinsic metacoxal muscles, Mx as abdominal muscle), compared to 27 in a more plesiomorphic pattern in other groups of Adephaga and in Polyphaga (Beutel & Haas, 2000; Larsén, 1966), and 36 in the archostematan *T. bruchi* (Friedrich et al., 2009). Compared to more generalized muscular patterns reported by Larsén (1966), M60 (IIIdlm1) M. metanoti primus, M61 (IIIdlm2) M. metanoti secundus, M63 (IIvlm5) M. metasterni secundus, M64 (IIIdvm1) M. dorsoventralis primus, M65 (IIIdvm8) M. dorsoventralis secundus, M66 (IIIdvm8) M. dorsoventralis tertius, M68 (IIItpm6) M. noto–pleuralis, M73 (IIIspm1) M. sterno–episternalis, M74 (IIIdvm2) M. noto–trochantinalis, M75 (IIIdvm4) M. noto–coxalis anterior, M77 (IIIpcm4) M. episterno–coxalis, M78 (IIIpcm3) M. coxa–basalaris, M79 (IIIdvm6) M. coxa–subalaris, M80 (IIscm7?) M. sterno–coxalis, M81 (IIIscm1) M. furca–coxalis anterior, M82 (IIIscm4) M. furca–coxalis lateralis and M83 (IIIscm2) M. furca–coxalis posterior are absent. Only two dorsoventral muscles M76 (IIIdvm5) and M84 (IIIdvm7) are present as indirect flight muscles. M67 (IIItpm2), M69 (IIItpm3), M70 (IIItpm10), and M71 (IIItpm7 & 9) are present as direct flight muscles. M85 (IIIscm6) is present, but the other furca–coxal muscles are absent.

4 | DISCUSSION

4.1 | Phylogenetic and evolutionary interpretations

Even though Gyrinidae are arguably the "basal" sister group of the remaining adephagan families and may have originated in the early Triassic or even the late Permian (Baca et al., 2017; Beutel & Roughley, 1988; Beutel et al., 2013; Ponomarenko, 1977), their morphology and life habits are distinctly modified compared to the hypothetical ground plan of the suborder and of Coleoptera (Beutel, 1997; Beutel & Haas, 2000; Friedrich et al., 2009). As is sometimes the case with so-called basal groups (e.g., Monotremata in mammals or Struthiones in birds; Mickoleit, 2004), the autapomorphies (Beutel, 1989a,b, 1990; Miller & Bergsten, 2012) outweigh few preserved plesiomorphies, such as the lack of the torsion of aedeagus or the retained intrinsic movability of the larval maxilla (Beutel & Roughley,

1988). The high number of gyrinid apomorphies mainly reflects adaptations to surface swimming in the case of adults, and a preference for greater water depths of the larvae, which are equipped with tracheal gills (Beutel & Roughley, 1988; Larsén, 1966).

The forelegs of Gyrinidae are long grasping devices suitable for seizing prey objects on the water surface, apparently an autapomorphy of the family (Beutel, 1989b). In contrast to most other groups of Adephaga, the ventral procoxal joint is reduced in Gyrinidae, with the exception of the "ancestral" *Spanglerogyrus* (Beutel, 1989b), increasing the degrees of freedom at the leg base. This character supports the monophyletic origin of the subfamilies Heterogyrinae (*Heterogyrus*) and Gyrininae (Beutel et al., 2017; Miller & Bergsten, 2012). Other features characterizing this clade are the presence of a prothoracic proprioceptive organ with spatulate setae (se: Figure 3c) and the laterally compressed protarsi (tar1: Figure 1b; 2a). In contrast to the pterothoracic segments, the musculature of the prothorax is plesiomorphic, with a well-developed set of neck muscles moving the head and normally developed leg muscles, similar to which in other groups of Coleoptera.

The mesothorax of Gyrinidae differs from all other Adephaga by the extensive and flat mesoventrite, which does not articulate with the prosternal process. Phylogenetically this character is ambiguous. Arguably it is a plesiomorphic trait compared with the short and grooved mesoventrite found in the other aquatic families, and also in the terrestrial Trachypachidae and Carabidae (partim) (Beutel, 1997; Beutel & Roughley, 1988). However, this structure is apparently suitable for gliding on the surface film of the water and more likely a secondarily acquired feature and autapomorphy of Gyrinidae.

The most conspicuous (and unique) synapomorphy of *Heterogyrus* and Gyrininae is the transformation of the middle and hind legs into shortened and flattened paddle-like structures (Figure 2b,c), with a fan-shaped tarsus. The swimming lamellae (Larsén, 1966), which do not occur in any other aquatic group of beetles, create 52% of the propulsion force (Nachtigall, 1961). These conditions are in contrast to the moderately modified middle and hind legs of Dytiscidae and *Spanglerogyrus* (Beutel, 1990; Nachtigall, 1960), which are more or less elongated and equipped with simple or feather-like swimming hairs, respectively. The paddle-like middle and hind legs enable whirligig beetles to swim rapidly on the water surface, with a frequency of the hind legs of about 60 strokes/sec and about 30/sec of the middle legs (Bott, 1928; Nachtigall, 1961). It was pointed out by Nachtigall (1961) that the paddle-like legs of Gyrininae exceed the performance of comparable technical machines and form the best-known thrust apparatus in the animal kingdom. The distal leg elements of *Gyrinus* investigated by Nachtigall (1961) are plesiomorphic compared with those of Orectochilini and most Enhydrini. The distal tarsomeres of Orectochilini and most enhydrine genera form a shovel-like structure with the basal ones (Beutel, 1990; Larsén, 1966), which likely improves the efficiency.

An unusual apomorphy of Orectochilini is the far-reaching reduction in the metathoracic muscular system. Compared with other groups of Neoptera, Coleoptera in general are characterized by a simplified pterothoracic muscular system, probably linked with the strong sclerotization without exposed membranes and reduced degrees of freedom, especially at the leg bases (Beutel & Haas, 2000; Friedrich et al., 2009). In contrast to more than 100 pterothoracic muscles suggested for the neopteran ground plan (Friedrich & Beutel, 2008), Larsén (1966) proposed 52 muscles as a plesiomorphic status of Coleoptera after a broad investigation of Adephaga and Polyphaga. More recent studies suggest that more muscles are present in the coleopteran ground plan (Beutel & Haas, 2000; Friedrich et al., 2009). 69 muscles were identified in *T. bruchi*, a species of Ommatidae in Archostemata, which is currently recognized as an evolutionary relict (Friedrich et al., 2009). Other thoracic plesiomorphies preserved in Ommatidae (and the closely related Cupedidae) are a transverse ridge of the mesoventrite and exposed metatrochantins (Baehr, 1975; Beutel & Haas, 2000; Friedrich et al., 2009). Within Orectochilini, only 22 pterothoracic muscles are preserved in *O. villosus*, 17 in *Gyretes zimmermanni* Ochs, 1929, and 16 in *Orectogyrus ornaticollis* Aubé, 1838 (Table 1; Larsén, 1966). Among them, 10 are preserved in the metathorax of *O. villosus* and 7 in the metathorax of the other two species examined by Larsén (1966). This is in agreement with a general trend of decreasing complexity of the thoracic musculature in Pterygota and in Coleoptera (Beutel & Haas, 2000; Friedrich & Beutel, 2010; Friedrich et al., 2009). The metathoracic muscular number observed in Orectochilini is the lowest among 54 beetles examined by Larsén (1966), also including flightless species if degenerated muscles are considered as present (marked as 0 in Larsén, 1966), and also less than those observed species in more recent studies on the thorax of Coleoptera (Baehr, 1975; Belkaceme, 1991; Beutel, 1986, 1988; Beutel & Komarek, 2004; Friedrich & Beutel, 2006). Derived skeletal features correlated with the muscular reductions exclude the interpretation that conditions observed in Orectochilini are due to slight intraspecific variation of the flight muscles. A conspicuous feature of Orectochilini (and the enhydrine genera) is the medially shortened metanotum (Beutel, 1990; Hatch, 1925; Larsén, 1966), which provides limited space for the dorsoventral muscles and no suitable attachment areas for dorsal longitudinal muscles. The loss of the metaphragma is apparently also linked with this modification.

4.2 | Functional interpretations of the locomotor apparatus

Coleoptera are characterized by reduced degrees of freedom in their thoracic skeleton, and also a distinctly reduced muscular system compared with other groups of Neoptera (Beutel & Haas, 2000) (Figure 6). The tendency to increase the efficiency and economy of the locomotor apparatus is intensified in the non-archostematan suborders, notably in Polyphaga and Myxophaga (Beutel & Haas, 2000). However, a culminating point is reached in the adephagan tribe Orectochilini of Gyrinidae, for instance *O. villosus*. Whirligig beetles in general have optimized surface swimming and retained the capacity of flight with a distinctly reduced pterothoracic muscle set (Larsén, 1966).

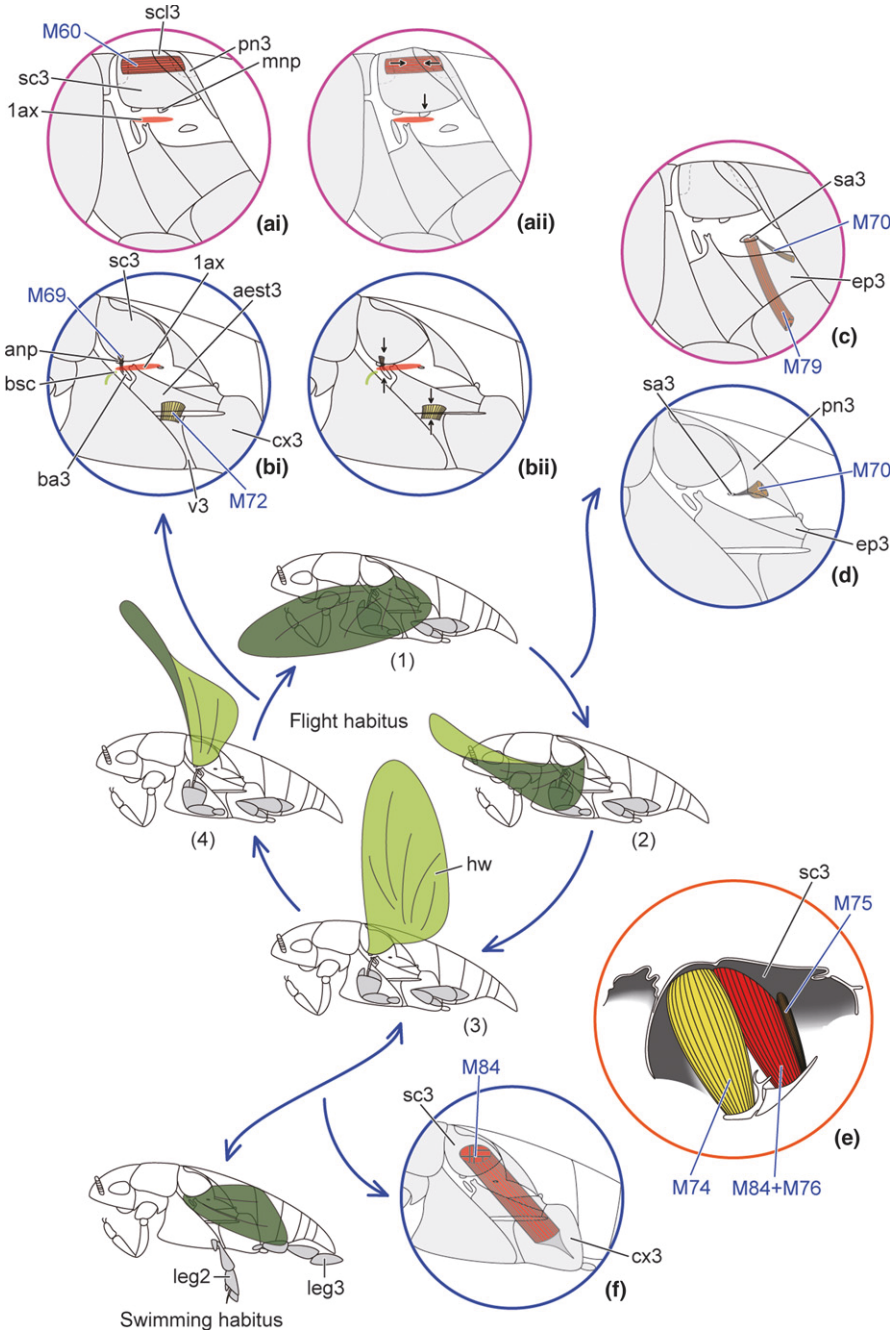

FIGURE 6 Schematic diagram for functional interpretation. The insect wing stroke can be divided into 4 distinct stages (Brodsky, 1994): (1) depression and turning forward; (2) turning backward and beginning supination; (3) elevation and end of supination; (4) pronation. In Gyrinidae, the mid- and hind legs (leg2, 3) are laid close to the body during flight (Larsén, 1966), and alternatively extended and flexed during swimming (Nachtigall, 1961). Elytra omitted. (ai, aii) M60 (IIIdlm1) initiates depression in generalized Coleoptera; (bi, bii) either M69 (IIItpm3) or M72 (IIIppm1) initiate depression in Gyrinidae; (c) both M70 (IIItpm10) and M79 (IIIdvm6) control supination in generalized Coleoptera; (d) only M70 (IIItpm10) controls supination in Gyrinidae; (e) the large metacoxal muscles nearly form a single compact unit in the cricket *Gryllus bimaculatus* (redrawn from Brodsky, 1994: figure 7.13a(ii)); (f) extremely large M84 (IIIdvm7) controls both hind wing elevation and hind leg backstroke

The dorsal longitudinal muscles are completely missing in Orectochilini and other groups of Gyrinidae (Larsén, 1966), apparently linked with modifications of the metanotum (Beutel, 1990). They are usually important elements of a flight apparatus operating with the neopteran (and ephemeropteran) indirect mechanism (Brodsky, 1994). The function of the metathoracic dorsal longitudinal muscle M60 (IIIdlm1) in Coleoptera is the longitudinal contraction of the notum, which results in the initial depression of the wings with the first axillary sclerite (Figures 6ai,aii; Brodsky, 1994; Haas & Beutel, 2001). This muscle is not only lacking in Orectochilini but also in *Heterogyrus* and Gyrininae (pers. obs. R.G. Beutel; Larsén, 1966). The intrinsic elasticity of the metanotum alone (Larsén, 1966) does not provide a sufficient explanation of the mechanism without adequate muscular control. Interestingly, the dorsal longitudinal muscles are not only reduced in Gyrinidae, but also poorly developed in two distantly related orders with posteromotorism, Orthoptera and Blattodea (Polyneoptera). In these cases, this is compensated by the metabasalar muscles according to Brodsky (1994). In most examined species of Gyrininae, the tergo–pleural muscle M69 (IIItpm3) is present and the only muscle attached to the metabasalare (Beutel, 1990; Larsén, 1966). Therefore, it is likely that this muscle takes over the function of hind wing depression (Figure 6bi,bii), with adduction and pronation of the hind wing through the metabasalare as additional functions (Brodsky, 1994). However, M69 (IIItpm3) is absent in most Orectochilini, and the metabasalare is solidly attached to the metanepisternum in species

of this tribe (Beutel, 1990; Larsén, 1966). We hypothesize that the functions are taken over by the metapleural muscle M72 (IIIppm1) which moves the sclerite, with a cleft forming a joint between the metanepisternum, the metaventrite, and the metacoxa (Figure 3d) (Larsén, 1966; Orectochilini and *Dineutus*). M72 (IIIppm1) is missing in *Gyrinus* and *Aulonogyrus* (Beutel, 1990; Larsén, 1954, 1966), whereas M69 (IIItpm3) is present in the species of both genera. Under the control of the metabasalare, the basisubcostale also becomes responsible for the depression (Brodsky, 1994). The control of the first axillary sclerite is shifted from the median notal process to the anterior notal process (Brodsky, 1994), which is generally preserved in Gyrinidae (Beutel, 1990; Hatch, 1926; Larsén, 1966).

The large metathoracic dorsoventral muscle M84 (IIIdvm7; in *O. villosus* length: 2.20 mm, width: 0.45 mm; length: width ratio = 4.9: 1.0) of Gyrininae (Larsén, 1966) must play a dominant role as a levator of the hind wing, considering the absence of most other dorsoventral muscles (Figure 6f; Larsén, 1966). Additionally, this muscle is responsible for the backstroke of the hind leg during swimming, as synergist of the large metacoxal muscle M87 (Figure 6f; Larsén, 1966; in *O. villosus* length: 1.80 mm, width: 1.69 mm). During this activity, the hind wings and associated elements of the flight apparatus are shielded and locked by the elytra, with the mesonotal scutellar shield and the metanotal alacristae forming a combined elytral arresting mechanism (Beutel, 1990; Beutel & Haas, 2000; Larsén, 1966). The activity of M84 (IIIdvm7) during flight is probably supported by another dorsoventral muscle, M76 (IIIdvm5). However, as this muscle is extremely slender (width 0.07 mm) compared with M84 (IIIdvm7), its effect is probably minimal.

In the ground plan of Coleoptera and in most species examined, two muscles connect with the metasubalare, the dorsoventral muscle M79 (IIIdvm6) and the tergo-pleural muscle M70 (IIItpm10) (Figure 6c; Larsén, 1954; Haas & Beutel, 2001; Friedrich et al., 2009; Friedrich & Beutel, 2010). Due to the absence or reduction in M79 (IIIdvm6) in Gyrinidae except *Dineutus* (Larsén, 1966), its function has to be taken over by another muscle (Figure 6d). It is conceivable that supination (Brodsky, 1994) is achieved by M70 (IIItpm10) in Orectochilini and most other Gyrinidae (Larsén, 1966).

It is noteworthy that the two bundles of the tergo-pleural muscle M71 (IIItpm7 and 9) are absent in the two orectochiline species *O. ornaticollis* and *G. zimmermanni* (Larsén, 1966), as in some other coleopteran species with reduced flight apparatus (Haas & Beutel, 2001). As the only muscle connected with the third axillary sclerite in Neoptera (Friedrich & Beutel, 2008), it is involved in at least three of the four stages of the wing stroke (Brodsky, 1994), also initiating the processes of unfolding and folding the wings (Brodsky, 1994; Haas & Beutel, 2001). We cannot exclude the possibility that these two orectochiline species are flightless. However, flight appears rather possible, as the hind wings and skeletal parts of their flight apparatus show no observable traits of reduction compared with the other Gyrinidae (Larsén, 1966). If indeed individuals of these two species are able to fly, it is unclear how the loss of M71 is compensated for.

It was pointed out that elytral movement of beetles plays a minor role in creating propulsive forces during flight (Haas & Beutel, 2001). Their obvious function is protecting the hind wings at rest, also shielding and locking the flight apparatus during swimming in Gyrinidae (Haas & Beutel, 2001; Larsén, 1966). Aside from this, they are probably also involved in flight control, improving the maneuverability of beetles and directing the airflow to the hind wings (Brodsky, 1994). It was demonstrated for species of Orthoptera that the control of posteromotoric flight is the most important function of the leathery forewings (Brodsky, 1994). The synchronous pronation and supination of coleopteran elytra during flight are probably not only a passive movement (Haas & Beutel, 2001), but also under the control of a series of mesothoracic flight-related muscles associated with the articulatory processes of the elytra (elap: Figure 3a).

The pterothoracic segments of Orectochilini differ strongly from conditions observed in other groups of Coleoptera (e.g., Belkaceme, 1991; Beutel, 1986, 1988; Beutel & Komarek, 2004; Friedrich et al., 2009; Larsén, 1966), with a remarkable degree of reduction in the muscular system. It is conceivable that this optimizes efficiency, especially in the context of dual alternative functions of flight and swimming on the water surface. Future investigations with biomechanical and physiological approaches may improve the understanding of the Orectochilini locomotor system, and possibly inspire interesting applications in bionics in the future.

ACKNOWLEDGEMENTS

We are very grateful to the editorial team, and to Dr. John Lawrence (Gympie, CSIRO Canberra) and Prof. Dr. Darren Pollock (Eastern New Mexico University) for their valuable comments. This project is financially supported by the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No. 642241.

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How to cite this article: Liu S-P, Wipfler B, Beutel RG. The unique locomotor apparatus of whirligig beetles of the tribe Orectochilini (*Gyrinidae*, *Coleoptera*). *J Zool Syst Evol Res*. 2017;00:1–13. https://doi.org/10.1111/jzs.12195

3.3. Study III

Liu, S.-P., Friedrich, F., Petersen, D.S., Büsse, S., Gorb, S.N. & Beutel, R.G. (2018)

The thoracic anatomy of the swift lousefly *Cratarina pallida* (Diptera) – functional implications and characters evolution in Hippoboscoidea. *Zoological Journal of the Linnean Society*, (https://doi.org/10.1093/zoolinnean/zly032).

Abstract

This manuscript describes the external and internal thoracic structures of swift lousefly *Crataerina pallida* (Diptera: Hippoboscidae) in detail, with a series of traditional and advanced morphological techniques. Compared with the other alate related species, the modified thoracic morphological results are discussed referring to flightlessness and ectoparasitism, including the obliterated dorsal segmental borders, the flattened thorax, the specialized leg and claw structures for clinging to the host and moving in the fur or plumage. 44 thoracic characters are used to reconstruct the evolution of Hippoboscoidea, which confirms the monophyly of Hippoboscoidea, Pupipara and bat flies. The genus *Ornithoica* with several plesiomorphic features challenges the monophyletic origin of Hippoboscidae.

3.3.1. Reviewed Version

The thoracic anatomy of the swift lousefly *Crataerina pallida* (Diptera) – functional implications and character evolution in Hippoboscoidea

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ABSTRACT

The thoracic skeletomuscular system of the swift lousefly *Crataerina pallida* (Diptera: Hippoboscidae) is described with SEM, μCT and 3D reconstruction. The morphological results are discussed with respect to ectoparasitism and flightlessness. The evolution of thoracic characters in Hippoboscoidea is reconstructed based on a parsimony analysis of 44 thoracic characters. The results confirm the monophyly of Hippoboscoidea, Pupipara (Hippoboscidae + Streblidae + Nycteribiidae) and the bat flies (Streblidae + Nycteribiidae). The monophyletic origin of Hippoboscidae is challenged with respect to the genus *Ornithoica*, which displays several plesiomorphic features compared with conditions shared by the remaining Pupipara. The thorax of most species in the three families is affected by ectoparasitism. The dorsal segmental borders are obliterated, and the thorax is usually strongly flattened. The legs are adapted to cling to the host and to move efficiently in the fur or plumage. A heel-like claw tooth supports large claws. Different reduction patterns of the flight apparatus occur in the group. Even though *Crataerina* and *Stenepteryx* are close relatives and similar in their general thoracic configuration, differences in the muscular patterning suggest independent losses of the flight capacity.

ADDITIONAL KEYWORDS: Hippoboscoidea–ectoparasite–flightlessness–thoracic morphology–evolution.

INTRODUCTION

Hippoboscidae, commonly known as louse flies or keds, are an exceptional group within the highly diverse Diptera. All species are parasitic and feed exclusively on fresh blood of homoiotherm vertebrates (Massonat, 1909; Bequaert, 1953; Maa & Peterson, 1987). This kind of specialized nutrition is rather rare among insects, but can be found in different groups of Diptera, including for instance "nematoceran" biting flies and black flies, but also tse-tse flies (Glossinidae) and bat flies (Nycteribiidae and Streblidae), which are close relatives of Hippoboscidae (Nycteribiidae and Streblidae where included in Hippoboscidae in alternative classifications; Griffiths, 1972; Pape & Thompson, 2018). Hippoboscidae combined with bat flies and tse-tse flies form the Hippoboscoidea. With the possible exception of Streblidae (Petersen *et al.*, 2007), monophyly is widely accepted for all family-level taxa and the superfamily as a whole (Hennig, 1973; McAlpine, 1989).

All Hippoboscoidea are adenotrophic viviparous (Meier, Kotrba & Ferrar, 1999). It has been postulated that high temperature in combination with sufficient nutrition, particularly haematophagy, are responsible for this kind of reproductive strategy (Roubaud, 1909; Senior-White, Aubertin & Smart, 1940). However, it is difficult to confirm this hypothesis as other insects living under similar conditions are not viviparous, and vivipary is not strictly associated with haematophagy (Meier *et al.*, 1999). In any case, hippoboscids apparently benefit from this reproductive adaptation: it results in a better protection of offspring from predation or parasitism, shortens the larval development and enables synchronization of hatching according to the presence of suitable hosts.

Shelter and forage, which are crucial for non-parasitic flies, are provided by the host in the life cycle of hippoboscids. However, reliable anchorage and efficient locomotion on the host become critical issues, especially for species with partly or completely reduced flight organs. The ectoparasitism requires far-reaching structural and physiological adaptations, for instance a flattened body and legs with specialized attachment devices (Kemper, 1951). Adaptive specialization of different body parts has likely led to an obligate dependence on a single host species in many cases (e.g. Bequaert 1953).

Various modifications of wings across hippoboscoid taxa is a striking feature of the group. The reduced wings probably correlate with an increased mobility on the host and is frequently observed among ectoparasites (Andersen, 1997). The horse ked *Hippobosca equinae* Linnaeus, 1758 has retained functional wings and is not restricted to a single host species. It also sucks blood from deer, dogs and humans (Hutson, 1984). In contrast, the deer fly *Lipoptena cervi* (Linnaeus, 1758), a poor flyer, sheds the wings after attaching to a suitable host (Hutson, 1984). Its host spectrum is restricted to deer and close relatives. The sheep ked *Melophagus ovinus* (Linnaeus, 1758) is entirely wingless, strongly associated with the domestic sheep and spends its entire life cycle on this host (Hutson, 1984).

The swift louse fly *Crataerina pallida* (Olivier in Latreille, 1812), which is in the focus of our study, displays an intermediate condition with regard to wing modification. The body is shortened and its reduced forewings are unsuitable for active flight (Hutson, 1984). Possible functions of the partly reduced wings are still under debate. Eichler (1939) suggested that they are used to perform small jumps, and Büttiker (1944) allegedly observed that they are used to glide for short distances in the air. Walker & Rotherham (2010) suggested that the wings might provide an additional anchorage, thus improving attachment to the host. However, this interpretation also requires confirmation. Thus, the actual function of the shortened wings still remains elusive.

C. pallida is an extremely agile, monoxenous avian ectoparasite of the common swift. Its success strongly depends on a permanent reliable contact with the host. The resulting structural and physiological adaptations make it a promising model for investigating evolutionary changes linked to parasitism. The common swift is a migrating bird with a high nest fidelity (Weitnauer, 1947; Lack & Lack, 1951) and is present in Europe only from May to September. The life cycle of *C. pallida* is adjusted to this seasonal appearance of the host. It places its fourth-instar larvae, which pupate immediately on emergence from the female, directly in the swift's nest to overwinter (Bequaert, 1953). The hatching of the pupae is temperature-sensitive and synchronized with the swift's return (Popov, 1965; Walker & Rotherham, 2010). Although *C. pallida* is strictly hematophagous and can remove up to 5% of the host's blood volume with a single bite, no detrimental effects on the host were found (Lee & Clayton, 1995; Tompkins, Jones & Clayton, 1996; Hutson, 1981; Walker & Rotherham, 2010), suggesting a reduction in virulence as a direct adaptation.

Apart from physiological and developmental adaptations (Meier *et al.*, 1999), structural modifications, especially in the thoracic segments, are apparently important for the success of this species. Swifts rely on their nests only for short periods, especially when breeding and feeding the offspring. Consequently, *C. pallida* must attach to the host and move across it efficiently both in the nest and during flight (Lack & Lack, 1951). Gustafson *et al.* (1977) and Henningsson *et al.* (2009) recorded that the swift can reach altitudes above 3500 m, and velocities distinctly exceeding 40 km/h. Consequently, reliable attachment of the ectoparasite under these conditions is essential (Kemper, 1951).

Despite the various fascinating aspects of the biology and morphology of *C. pallida*, external and internal structures of the thorax are insufficiently known (Massonat, 1909). Here a detailed description of the thoracic skeletomuscular system is provided using an array of modern techniques. The morphological results are discussed with respect to functional adaptations to the specialized lifestyle, with a special focus on wing reduction in *C. pallida* and related taxa. The character evolution in Hippoboscoidea is reconstructed based on a matrix of thoracic characters, which is analyzed using parsimony. Finally, a table homologizing thoracic muscles of different representatives of Diptera is presented.

MATERIALS AND METHODS

One specimen of *C. pallida* was manually dissected in 70% ethanol under a Zeiss Stemi SV 11 with an additional Euromex Illuminator EK-1 lighting system (Carl Zeiss MicroImaging GmbH, Oberkochen, Germany). The line drawings were executed with a pencil under the microscope, scanned and finished with Adobe Illustrator CC (Adobe Systems, San Jose, California, USA). Sclerites and body margins were drawn with full lines, margins below other sclerites and folds at the wing base with dotted lines. Wings and legs were omitted, except basal elements, i.e. coxae, trochanters and wing base sclerites.

For µ-CT analysis, the samples were dehydrated in an ascending ethanol series, critical point dried with a Quorum E3000 (Quorum Technologies Ltd, Laughton, UK) and scanned using a Skyscan 1172 (Bruker micro-CT, Kontich, Belgium) desktop µ-CT. The scans were performed with 40 kV voltage, 250 µA current, 720 ms exposure and a rotation of 360° in steps of 0.25°. Images were taken at a resolution of 2.2 µm per voxel. The µ-CT data were reconstructed with FEI Amira 6.0 (FEI, Mérignac, France). Segmented structures were exported as stacks of tiff flies into Volume Graphics VGStudiomax 2.0 (Volume Graphics, Heidelberg, Germany), which was used for volume rendering and filming. The quality of the µ-CT result was not sufficient for a clear visualization with a 3D reconstruction. Therefore, the final skeletal-muscular figures were drawn with Adobe Illustrator CC (Adobe System, San Jose, California, USA) based on the reconstructions and direct observation using the dissected specimens.

For SEM analysis, the samples were dehydrated in an ascending ethanol series, critical point dried with a Quorum E3000 (Quorum Technologies Ltd, Laughton, UK) and sputter-coated with gold-palladium (10 nm thickness; Leica Bal-TEC SCD500 (Leica Microsystems GmbH, Wetzlar, Germany)). Afterwards the samples were mounted on a rotatable sample holder (Pohl, 2010) and examined using a Hitachi TM3000 (Hitachi High-Technologies Corp., Tokyo, Japan) scanning electron microscope at an accelerating voltage of 15 kV.

The terminology for the thoracic skeleton follows Schlein (1970) and Friedrich & Beutel (2010a), for the wing base elements Fabian, Schneeberg & Beutel (2016), for distal leg structures Friedemann, Schneeberg & Beutel (2014) and for the chaetotaxy Maa & Peterson (1987) and Fabian *et al.* (2016). The dipteran thoracic muscular names used by Maki (1938), Bonhag (1949), Smart (1959), Christophers (1960), Nußbaum (1960), Mickoleit (1962), Schlein (1970), Ulrich (1971, 1984), Owen (1977) and Fabian *et al.* (2016) were homologized with the generalized neopteran muscular nomenclature of Friedrich & Beutel (2008). The muscle names of Friedrich & Beutel (2008) were also used for the description of the thorax of *C. pallida*.

Some additional dried specimens at Naturhistorisches Museum Wien were examined, which include: **Glossinidae:** *Glossina* spp.; **Hippoboscidae:** *Ornithoica*

pusilla (Schiner, 1868), *Ornithomyiia avicularia* (Linnaeus, 1758), *Lipoptena cervi* (Linnaeus, 1758) and *Melophagus ovinus* (Linnaeus, 1758); **Streblidae:** *Nycteribosca kollari* Frauenfeld, 1855; **Nycteribiidae:** *Eucampsopoda hyrtli* Kolenati, 1856, *Nycteribia allotopa* (Speiser, 1901), *Cyclopodia horsfieldi* de Meijere, 1899, *Stylidia hermanni* Leach, 1816.

Forty four thoracic morphological characters were coded and entered in a data matrix for 8 taxa and 2 outgroups (Table 1). The parsimony analysis was carried out with WinClada 1.00.08 and NONA 2.0 software (Goloboff, 1999; Nixon, 1999-2002) and TNT 1.1 (Goloboff, Farris & Nixon, 2008). Bremer support values (Bremer, 1994) were calculated with NONA 2.0.

ABBREVIATIONS

a/m/pwp – anterior/median/posterior notal wing process; ax1/2/3/4 – first/second/third/fourth axillary sclerite; ba2 – mesobasalare; bc – basicosta; bs1/2/3 – pro/meso/metabasisternum; cl – claw; cp – cryptosternum; cx1/2/3 – pro/meso/metacoxa; clt – claw tooth; ec – mesepisternal cleft; em1/2/3 – pro/mes/metepimeron; epd – empodium; es1/2/3 – pro/mes/metepisternum; fu1/2/3 – pro/meso/metafurca; fs2/3 – meso/metafurcasternum; h – haltere; hc – humeral callus; lcv1/2 – first/second lateral cervical sclerite; leg1/2/3 – fore/mid/hind leg; mer2/3 – meso/metacoxal meron; np – notopleural suture; nt1/3- pro/metanotum; ph1 – prophragma; plr2 – mesopleural ridge; pls1/2/3 – pro/meso/metathoracic pleural suture; pnc – postnotal calli; pu – pulvillus; pwp2 – mesothoracic pleural wing process; scl2 – mesoscutellum; scs – subcoxal sclerite; sl – sensillar area; tr1/2/3 – pro/meso/metatrochanter; ts – transverse suture; sa2 – mesosubalare; sp1/3 – pro/metathoracic spiracle; tg – tegula. DC – post-sutural setae of dorsal central region; NP – notopleural setae; PA – post-alar setae; PP – postpronotal setae; S – scutellar setae; SA – supra-alar setae.

RESULTS

THORACIC SKELETON

General features

The thoracic segmental borders appear indistinct in dorsal view. The entire thorax is flattened, ca. 1/3 high as wide. The mesothoracic region is distinctly widened. The dorsal side is largely sclerotized. The head and thorax are tightly connected, together forming a streamlined compact functional unit; the posterior part of the head covers the dorsal prothorax completely. The sclerotized dorsal parts of the thorax and the tarsi are brown, whereas the other regions and the proximal parts of the legs display a pale yellowish coloration.

Cervix and prothorax

The cervix and prothorax are dorsally covered by the occipital region of the head. The small triangular first lateral cervical sclerite (lcv1: Fig. 4A) connects the dorsal part of the second lateral cervical sclerite with its tapering posterior part. The second lateral cervical sclerite (lcv2: Figs. 4A; 5D) provides a large surface area for the attachment of dorso-ventral muscles; in anterior view, it appears curved and narrow; it is not supported by the proepisternum. A pair of tiny sclerotized sensillar areas (sl: Fig. 4A) are located in the membranous area between the ventral parts of the second lateral cervical sclerites.

The very narrow pronotum (nt1: Figs. 4A, B) is concave antero-medially and expanded laterally; it articulates with the lateral margin of the second lateral cervical sclerites with its sharp ventro-mesal edge. The unpaired prophragma (ph1: Fig. 5D) is suspended below the strongly sclerotized posterior pronotal margin. Very distinct and prominent humeral calli (hc: Fig. 4B) are present at the mesonotal antero-lateral corners; they extend anterad and fit tightly with the postero-lateral head capsule. A conspicuous, elongated and dorsally oriented spiracle is present posteriorly (sp1: Figs. 2A, C; 4B, F). The small propleuron is inserted between the humeral callus dorsally and the procoxa (cx1: Figs. 2B, C; 4A, D, F; 5D) on the ventral side. The slightly curved propleural suture (pls1: Fig. 4F) divides the sclerite into the anterior proepisternum (es1: Fig. 4F) and the posterior proepimeron (em1: Fig. 4F). Both articulate with the postero-dorsal procoxal margin with their ventral parts. A large membranous area is inserted between the inner margins of the large procoxae; dorsally connected with the second lateral cervical sclerite; posteriorly it is connected with the well-developed paired probasisterna (bs1: Figs. 4A, D), which are placed between the postero-mesal procoxal edges. Medially a broad probasisternal apodeme (ma: Fig. 5C) extends postero-dorsad. Close to its basal part, a slender and curved profurca (fu1: Fig. 5D) extends postero-dorsad; it is connected with the propleural suture.

Mesothorax

The very broad mesoscutum (sc2: Figs. 2A; 4A, B) is longitudinally subdivided by a distinct median suture. A pair of transverse sutures (ts: Fig. 4B) on the posterior mesoscutal third divides the sclerite partially in transverse direction. The notopleural suture is distinct and slightly curved (np: Figs. 2C; 4B); it extends from the lateral edge of the middle region of the mesoscutum to the mesopleuron. The flat triangular mesoscutellum (scl2: Figs. 2A; 4B, C) is attached to the posterior mesoscutal margin; its lateral arm is extended to form the posterior margin of the wing base. The postnotum (pn2: Fig. 4C) appears as a curved transverse band below the posterior scutellar edge. Laterally a pair of large bulges forms the postnotal calli (pnc: Figs. 2C; 4C, F).

The mesepisternum is very large (es2: Figs. 2C; 4F); a broad anterior process connects it with the posterior proepimeral margin; dorsally a long and curved suture

separates it from the humeral callus and mesoscutum. The mesobasalare (ba2: Figs. 2C; 4F) extends from the postero-median mesepisternal margin; it is enlarged dorsally; a distinctly developed basalar apodeme is lacking. The curved mesothoracic pleural suture (pls2: Figs. 2C; 4F) separates the mesepimeron (em2: Figs. 2C; 4C, F) from the anterior mesepisternum (es2: Figs. 2C; 4F). The former postero-dorsally connects with the lateral postnotal margin. The mesepisternal cleft (ec: Fig. 4F) also separates the mesepimeron from the anterior mesobasalare. Dorsally the mesothoracic pleural wing process (pwp2: Figs. 2C; 4F) is present as a tiny process, close to the dorsal mesosubalare (sa2: Fig. 4F). The triangular mesopleural ridge (plr2: Fig. 5D) extends along the mesopleural suture above the mesocoxal rim. The mesopleural suture ventrally articulates with the elongated mesocoxa (cx2: Figs. 2B, C; 4D, F) and mesomeron (mer2: Figs. 2B, C; 4D, F). Together these proximal leg elements form a wide articulatory area for the mesotrochanter (tr2: Fig. 4D).

The mesobasisternum (bs2: Figs. 2B; 4D) is large and broadly fused with the mesepisterna laterally. The elongated triangular cryptosternum (cp: Fig. 5C) extends into the thoracic lumen from the external median suture; its posterior part is divided into two branches, each of them connected to the mesofurca on the respective side. The mesofurcasternum (fs: Fig. 4D) is a small area behind the mesobasisternal posterior corner. The strongly developed mesofurca (fu2: Fig. 5D) extends along the mesobasisternal posterior margin; dorsally it forms an apical disc; laterally a thick, curved arm connects it to the mesepimeron.

Metathorax

The metanotum (nt3: Fig. 4C) is very small compared with the mesonotum and widely separated into two parts by the median intersegment between the thorax and abdomen. Both metanotal halves are dorsally fused with the ventro-lateral postnotal margin. Each of them is almost completely divided by a deep, vertically oriented concavity where the halteres are inserted. The halteres (h: Figs. 2C; 4C, F) are distinctly developed, with a conical base, a short stalk and an oval knob-like distal part. The metepisternum is dorsally connected with the ventral metanotal margin and is ventrally narrowing towards to metacoxal rim. Anteriorly the metathoracic spiracle (sp3: Fig. 4C) is embedded in the membranous area below the postnotal callus. It is shorter than its prothoracic counterpart. The curved metathoracic pleural suture (pls3: Fig. 4C) divides the metepimeron (em3: Fig. 4C) into two parts. The laterally extended ventral part reaches the metacoxal rim, and a thin mesal projection is medially fused to the corresponding metepimeral element of the other side. The very large metacoxae include a well-developed metameron (cx3 + mer3: Figs. 2B; 4D). The anterior margin of the wide metabasisternum (bs3: Figs. 2B; 4D) projects into the posterior concavity of the mesobasisternum. Its posterior area is completely fused with the metafurcasternum (fs3: Figs. 4C, D). The large triangular metafurca (fu3: Fig. 5C) extends along the median line; its antero-median process is attached to the postero-median margin of the cryptosternum.

Legs

The legs are strongly developed. The distal elements of all three pairs are similar (see hindleg: Figs. 3A, B, C), but the coxae differ distinctly in shape, size and articulation. The procoxae (cx1: Figs. 2B, C; 4A, D, F; 5D) appear inflated and are freely movable in all directions; a distinct, slightly rounded vertical edge is present antero-laterally; a dense field of short spines is inserted on the anterior margin and anterior surface. The roughly triangular protrochanter (tr1: Figs. 2B; 4A, D) is inserted on a wide membranous area of the distal procoxal part; its distal part is movably connected with the profemoral base. The profemur is almost straight, with nearly parallel anterior and posterior margins and an oblique distal edge; it bears a distinct vestiture of longer setae, mainly concentrated on the distal part, and shorter setae on the anterior area of the proximal region. The protibia is about as long as the profemur, narrower, slightly curved basally and widening distally; the vestiture of setae on the protibia is sparse compared with that of the profemur; a row of long setae is present on the middle region of the dorsal surface; shorter setae are inserted along the anterior margin. The protarsomeres 1-4 are short and moderately flattened; protarsomere 4 is apically emarginated; protarsomere 5 is large, about as long as 1-4 combined, also flattened, and distinctly widened distally; all protarsomeres bear area of short setae; two extensive setose fields cover almost the entire surface of protarsomeres 1-4, leaving only a narrow stripe on the dorsal and ventral side glabrous; protarsomere 5 bears only three pairs of setae on the dorsal surface, one pair at the edges and two closely adjacent pairs on the middle region of the distal edge; very few setae are inserted on the lateral surface and two fields of setae are present on the ventral side: one on the ventro-lateral edges and one apically close to the empodium. The empodium is about half as long as protarsomere 5, slender and slightly narrowing distally; rows of very short setae are inserted along the edges and on the ventral side of the distal 2/3; all setae are directed towards the apex; the base of the empodium is glabrous. The pulvilli are flexible structures, covered with minute hairs (Richards & Richards, 1979: acanthae) on the ventral surface. The claws are strongly developed, both forming curved bifid structures, appearing tridentate together with the strongly developed curved and heel-like claw tooth; very fine grooves are discernable on the internal surface of the claws.

The mesocoxae (cx2: Figs. 2B, C; 4D, F) are smaller than their prothoracic counterparts and restricted in their movability; in contrast to Schlein (1970) they contain a narrow sclerotized mesomeron (mer2: Figs. 2B, C; 4D, F). The metacoxae (cx3: Figs. 2B, C; 4D) contain a large metameron (mer3: Figs. 2B; 4D); they are distinctly larger than the procoxae and largely or completely immobilized at their base. The meso- and metafemora (metafemur: Figs. 3A, B, C) are slightly curved posterad, and slightly more elongated than their prothoracic counterparts. The tibiofemoral joint of the midleg is more strongly pronounced than that of the foreleg, and even more

conspicuous on the hind leg. The metatibiae are slightly more elongated than the pro- and mesotibiae.

Mesothoracic wing (Fig. 2D) and wing base (Fig. 4E)
The lancet-shape forewings are distinctly shortened, measuring 59% of the total body length from the base to the apex in males and 77% in females (Massonat, 1909: fig. 16). The venation is distinctly modified, concentrated in the anterior region of the proximal half. Four longitudinal veins reach the hind margin. Only two cross veins are present.

The wing base sclerites are largely merged with the membranous dorso-lateral region of the segment. Consequently, their shape and configuration are difficult to trace. The slightly bulging mesothoracic tegula (tg) is distally surrounded by the basicosta (bc) and the narrow subcoxal sclerite (scs). The triangular first axillary sclerite (ax1) proximally articulates with the blunt anterior notal wing process (awp), and also with the median notal wing process (mwp). Distally the second axillary sclerite (ax2) articulates with the anterior margin of the elongated third axillary sclerite (ax3). The proximal margin of the latter is close to the narrow fourth axillary sclerite (ax4). The posterior notal wing process (pwp) is slender proximally.

Metathoracic haltere
The short stalks of the spoon-shaped halteres (h: Figs. 2C; 4C, F) are located in a membranous area surrounded by the posterolateral parts of the metanotum.

Chaetotaxy
The postpronotal (PP: Figs. 2A; 3A, B) and notopleural setae (NP: Figs. 2A; 4B) form a continuous cluster from the humeral callus to the mesonotal antero-lateral area. The wing base is surrounded by the supra-alar (SA: Figs. 2A, C; 4B) and post-alar setae (PA: Figs. 2A; 4B); the former also extend to the dorsal mesepisternal area. Only 3-4 post-sutural dorsal setae (DC: Figs. 2A; 4B) are present in the central area between the transverse suture and the mesonotal posterior margin. The scutellar setae (S: Figs. 2A; 4B) densely cover the entire mesoscutellum.

THORACIC MUSCULATURE

Prothorax
Dorsal longitudinal muscle: **Idlm1** M. prophragma-occipitalis: O (=origin): prophragma; I (=insertion): dorsal area of occipitale.

Dorsoventral muscles: **Idvm1** M. cervico-occipitalis anterior, conical, broader at attachment area on cervical sclerite, narrowing towards insertion on head capsule, O: antero-ventral area of second lateral cervical sclerite; I: postero-median area of occipital region of head capsule. **Idvm2** M. cervico-occipitalis medialis, slightly bent upwards, narrowing towards insertion, O: postero-dorsal area of second lateral

cervical sclerite; I: postero-median area of occipital region. **Idvm3** M. cervico-occipitalis posterior, narrowing towards occipital, O: postero-dorsal area of second lateral cervical sclerite; I: postero-median area of occipital region. **Idvm5** M. pronoto-cervicalis anterior, O: median area of pronotum; I: antero-dorsal area of second lateral cervical sclerite. **Idvm7** M. pronoto-cervicalis posterior, O: postero-median pronotal margin; I: postero-dorsal area of second lateral cervical sclerite. **Idvm15** M. pronoto-trochantinocoxalis, very large, broader on area of origin, narrowing towards insertion: O: antero-median area of mesoscutum; I: antero-dorsal procoxal rim. **Idvm18** M. pronoto-coxalis lateralis, very large, broader on area of origin, narrowing towards insertion, O: meso-lateral area of mesoscutum; I: postero-dorsal procoxal rim. **Idvm19** M. pronoto-trochanteralis, very large, broader on area of origin, narrowing towards insertion: O: anterior half area of mesoscutum; I: protrochanteral tendon.

Ventral longitudinal muscles: **Ivlm1** M. profurca-cervicalis, very short, O: ventro-distal area of profurca; I: postero-ventral area of second lateral cervical sclerite. **Ivlm3** M. profurca-tentorialis, triangular, broader on profurcal area of origin, narrowing towards insertion on head capsule, O: dorsal side of dorsal area of profurca; I: posterior occipital area. **Ivlm7** M. profurca-mesofurcalis, broad, O: ventral side of dorsal area of profurca; I: antero-median area of mesofurca.

Sterno-coxal muscles: **Iscm1** M. profurca-coxalis anterior, large, O: dorsal area of median apodeme of probasisternum; I: anterior procoxal rim. **Iscm2** M. profurca-coxalis posterior, broadly triangular, broader on profurcal area of origin, narrowing towards insertion, O: ventro-proximal area of profurca; I: postero-dorsal procoxal rim. **Iscm3** M. profurca-coxalis medialis, slightly bent, O: proximal region of profurca, close to the base; I: antero-ventral procoxal rim. **Iscm4** M. profurca-coxalis lateralis, wide, triangular, broader on profurcal area of origin, narrowing towards insertion, O: ventral side of distal part of profurca; I: postero-dorsal procoxal rim. **Iscm6** M. profurca-trochanteralis, broader on area of origin, narrowing towards insertion: O: dorsal side of upper part of profurca; I: protrochanteral tendon.

Mesothorax

Dorsal longitudinal muscles: **IIdlm1** M. prophragma-mesophragmalis, narrow on postnotal callus, very broad on mesoscutum: O: posterior half area of mesoscutum; I: postnotal callus. **IIdlm2** M. mesonoto-phragmalis: O: postero-lateral area of mesoscutum; I: postnotal callus.

Tergo-pleural muscles: **IItpm1** M. prophragma-mesanepisternalis, broader on mesepisternum, narrowing towards mesoscutum: O: postero-lateral area of mesoscutum; I: postero-median area of mesepisternum m. **IItpm4** M. mesonoto-pleuralis anterior, small: O: postero-median area of mesopleural ridge; I: first axillary sclerite. **IItpm6** M. mesonoto-pleuralis posterior, short and thick: O: postero-dorsal area of mesopleural ridge; I: postero-lateral mesoscutal margin. **IItpm7** M. mesanepisterno-axillaris: O: postero-median area of mesepisternum; I: third axillary sclerite. **IItpm9** M. mesepimero-axillaries tertius, conical, broader on mesepimeron,

narrowing towards third axillary sclerite: O: mesepimeron close to base of mesopleural ridge; I: third axillary sclerite.

Sterno-pleural muscles: **IIspm2** M. mesofurca-pleuralis, slightly bent, O: distally on dorsal side of mesofurca; I: dorsal part of mesothoracic pleural suture.

Pleuro-coxal muscles: **IIpcm4** M. mesanepisterno-coxalis posterior, very large, broader on area of origin, narrowing towards insertion, O: anterior area of mesopleural ridge; I: antero-ventral mesocoxal rim. **IIpcm6** M. mesopleura-trochanteralis, very large, narrowing towards insertion, O: antero-dorsal area of mesopleural ridge; I: mesotrochanteral tendon.

Ventral longitudinal muscle: **IIvlm3** M. mesofurca-metafurcalis, conical, broader on metafurca, narrowing towards mesofurca, O: postero-dorsal area of mesofurca; I: dorsal tip of metafurca.

Sterno-coxal muscles: **IIscm1** M. mesofurca-coxalis anterior, very large, broader on are of origin, narrowing towards insertion, O: anterior area of cryptosternum; I: antero-ventral mesocoxal rim. **IIscm2** M. mesofurca-coxalis posterior, very large, narrowing towards insertion: O: median area of cryptosternum; I: mesocoxal posterior rim. **IIscm3** M. mesofurca-coxalis medialis, broader on are of origin, narrowing towards insertion, O: postero-median area of mesobasisternum; I: ventral mesocoxal rim. **IIscm6** M. mesofurca-trochanteralis, strongly developed, O: dorso-distal area of mesofurca; I: mesotrochanteral tendon.

Metathorax

Tergo-pleural muscles: **IIItpm4** M. metanoto-pleuralis anterior, O: metepisternum; I: basally on haltere. **IIItpm9** M. metepimero-axillaris tertius, O: dorsal part of metepimeron; I: basally on haltere.

Pleuro-coxal muscle: **IIIpcm2** M. metabasalare-trochantinalis, O: metepisternum; I: antero-dorsal metacoxal rim.

Sterno-coxal muscles: **IIIscm1** M. metafurca-coxalis anterior, very strongly developed, O: antero-median process of metafurca; I: antero-ventral metacoxal rim. **IIIscm2** M. metafurca-coxalis posterior, very large, O: antero-dorsal area of metafurca; I: postero-dorsal metacoxal rim. **IIIscm3** M. metafurca-coxalis medialis, broader on area of origin, narrowing towards insertion, O: postero-ventral area of metafurca; I: postero-ventral metacoxal rim. **IIIscm6** M. metafurca-trochanteralis, two large bundles, O: antero-dorsal metafurcal margin and median area of metabasisternum; I: metatrochanteral tendon.

Due to the strongly reduced metathorax, the homology of the three pleural muscles (IIItpm4, IIItpm9, IIIpcm2) is difficult to assess. Our provisional homology assessment is based on their relative positions.

PHYLOGENETICALLY RELEVANT CHARACTERS OF THE THORAX

In the following section, thoracic characters of *C. pallida* and other representatives of Hippoboscoidea are listed and discussed. Based on the observations presented here and published morphological information (e.g. Massonat, 1909; Bequaert, 1953; Schlein, 1970) the characters are coded for 8 selected hippoboscoid taxa and 2 outgroup terminals with available detailed data. The characters where analysed cladistically as outlined in Material and methods.

General thoracic characters

1. Thoracic segmental borders on dorsal side: (0) distinctly separated segments; (1) indistinct; (2) segmentation almost completely obliterated. A characteristic feature of Pupipara is the far-reaching obliteration of the dorsal segmental borders of the thorax. (Massonat, 1909: "aucune trace de segmentation", pls. I-VII; Bequaert, 1953; Zeve & Howell, 1963: figs 1-6; Schlein, 1970). The three segments form a compact ensemble, also allowing unusual shifts of muscles. The dorsal thoracic division is less indistinct in *Ornithoica* (Ornithoicinae) than in other hippoboscids (Bequaert, 1953).
2. Dorsoventral compression of thorax: (0) not flattened; (1) moderately flattened; (2) strongly flattened, height less than 1/2 width of mesonotum. Usually distinctly flattened in Pupipara, often only one third as high as mesonotal width (Massonat, 1909; Bequaert, 1953: fig. 8A; Schlein, 1970; Maa & Peterson, 1987; Wenzel & Peterson, 1987). Less compressed dorsoventrally in *Ornithoica* (Bequaert, 1953: "less depressed than that of the other subfamilies…"; Hennig, 1973). Varying in Streblidae from subglobose to strongly flattened (Streblinae) or laterally compressed and flea-like (Nycteribiinae) (Wenzel & Peterson, 1987). About as high as mesonotal width in Glossinidae (Bequaert, 1953: fig. 8B) and other groups of Diptera (Hennig, 1973).

   Schlein (1970) suggested a correlation between flattened thoracic segments and bent mesothoracic pleural sutures with two sharp edges. However, this interpretation is questionable. A similar feature also occurs in other dipteran families with a largely unmodified thorax (Bonhag, 1949: Tabanidae; Schlein, 1970: Muscidae).
3. Sclerotization of dorsal thoracic surface: (0) largely or completely sclerotized; (1) rudimentary. A largely unsclerotized thoracic dorsum (Nußbaum, 1960: fig. 2; Schlein, 1970) is likely an autapomorphy of Nycteribiidae.
4. Connection of head and thorax: (0) both not forming a tight unit; (1) head fitting tightly into thorax, both forming a streamlined unit. The head of *Crataerina* and *Stenepteryx* fits tightly with the anterior thorax, both forming a compact, streamlined unit (Bequaert, 1953). A close cephalo-thoracic connection is characteristic for ectoparasites of birds and is most perfect in species using fast-flying swallows or swifts as hosts.

Cervix and prothorax

5. Sensillar area of cervical region: (0) one plate; (1) two plates. Two plates are present in the membranous area between the ventral parts of the second lateral cervical sclerites of *Crataerina*. Two are also present in *Hippobosca* and *Musca*, but only one in Glossinidae, Streblidae and Nycteribiidae (Schlein, 1970).
6. First lateral cervical sclerites: (0) present; (1) absent. Normally present but missing in *Nycteribosca* and other Streblidae, and also in Nycteribiidae (Bequaert, 1953; Hennig, 1973; Schlein, 1970).
7. Proepisternal support of second lateral cervical sclerite: (0) present; (1) reduced. The pleural support of the second cervical sclerite is reduced in *Nycteribosca* and other Streblidae (Hennig, 1973; Schlein, 1970). It is present in *Hippobosca* but absent in other Hippoboscidae including *Crataerina*. Also present in *Musca* (Schlein, 1970).
8. Protergum: (0) normally developed; (1) strongly shortened. Generally strongly shortened in Pupipara (Bequaert, 1953).
9. Space between procoxae: (0) procoxae adjacent or nearly adjacent medially; (1) separated by ca. 1/3 of coxal width, without large membranous area; (2) widely separated, with large membranous area between them. Usually more or less contiguous in Diptera (Hennig, 1973). Moderately widely separated in Glossina (Bequaert, 1953: fig. 9 B). Widely separated in Pupipara (Bequaert, 1953: fig. 9a). Narrowly separated in Glossinidae.
10. Probasisternum: (0) distinctly developed; (1) largely reduced or absent as a recognizable separate structure. Vestigial in Streblidae and largely or completely reduced in Nycteribiidae (Schlein, 1970).
11. Median apodeme of probasisternum: (0) absent; (1) present. A median apodeme is present in *Glossina*, *Hippobosca* and *Crataerina*. It is missing in *Drosophila* and *Musca*, and also in Streblidae and Nycteribiidae (Schlein, 1970).
12. Tube-like sensory organ of prosternal region: (0) absent; (1) present. Present in Nycteribiidae (Schlein, 1970).

Mesothorax

13. Shape of mesothorax: (0) as long as or longer than wide; (1) wider than long. Almost generally wider than long in Pupipara (Massonat, 1909: pls. I-VI; Bequaert, 1953: fig. 8A), but laterally compressed in some Streblidae (Wenzel & Peterson, 1987: Nycterophiliinae). Longer than wide in Glossinidae (Bequaert, 1953: fig. 8B).
14. Shape of humeral callus: (0) not distinctly protruding; (1) distinctly protruding and pointed. Very prominent in *Crataerina* and other Ornithomyiinae (Hennig, 1973), but indistinct in *Ornithoica* (Hennig, 1973) and Lipopteninae,

and slightly projecting in *Hippobosca* (Bequaert, 1953). The posthumeral border of the humeral callus is obliterated in Streblidae (Schlein, 1970).

Even though the humeral calli and the adjacent spiracles are completely fused to the mesonotum, they are likely derived from the posterior pronotal area. This is suggested by a comparison with the pronotal subdivision in Tipulidae (Matsuda, 1970), and also by postpronotal setae covering the humeral calli.

15. Median suture dividing mesonotum: (0) absent; (1) present. Present and dividing mesonotum in *Crataerina* and most other Hippoboscidae, and also distinct in Streblidae (Massonat, 1909: figs. 24, 30, 33, 36, 40, 46; Bequaert 1953: fig. 8A; Zeve & Howell, 1962: figs 1, 2). Short in some species of *Lipoptena* (Bequaert, 1953: fig. 11A) and indistinct but still recognizable in *Stenepteryx* (Massonat, 1909: fig. 54). Absent in Nycteribiinae (Peterson & Wenzel, 1987) and Glossinidae. Absent in *Melophagus* (Massonat, 1909: fig. 20) and *Ornithoica* (Maa, 1966: figs 4, 5). Inapplicable (coded as [-]) in Nycteribiidae due to unsclerotized thoracic dorsum (Nußbaum, 1960).
16. Mesepisternal ridge: (0) developed; (1) reduced. The mesepisternal ridge is reduced in *Glossina* and Pupipara (Schlein, 1970).
17. Space between mesocoxae: (0) narrowly separated; (1) widely separated. Very widely separated in Pupipara (Hennig, 1941; Bequaert, 1953: fig. 9a; Schlein, 1970: figs 23, 28A). Narrowly separated in Glossinidae.
18. Size of mesobasisternum: (0) moderately sized; (1) greatly expanded. Very extensive in *Crataerina* and other groups of Pupipara (Bequaert, 1953; Schlein, 1970).
19. Anterior end of mesobasisternum: (0) not folded upwards and backwards; (1) folded upwards and backwards. Folded upwards and backwards in Streblidae and Nycteribiidae, displacing the profurca on a second plane (Schlein, 1970).
20. Mesofurca: (0) well developed; (1) reduced. The mesofurca is reduced to a small stalk in Nycteribiidae (Schlein, 1970).
21. Cup-shaped upper end of mesofurca: (0) present; (1) absent. The cup-shaped upper end of the mesofurca, which normally serves for attachment of flight muscles, is missing in Nycteribiidae (Schlein, 1970).

Metathorax

22. Transverse suture of metanotum: (0) present; (1) small triangular sclerites above halteres; (2) with two lateral posterior lobes. Metanotum represented by transverse suture in Hippoboscidae (Schlein, 1970) and *Drosophila* (Fabian *et al*., 2016), but by small triangles above the halteres in *Musca* and *Glossina*.With two lateral posterior lobes in *Nycteribosca* (Streblidae) (Schlein, 1970).
23. Ventral connection of metepimera: (0) separated; (1) fused ventrally. Fused ventrally in Hippoboscidae, Streblidae and Nycteribiidae (Schlein, 1970).

24. Space between metacoxae: (0) narrowly separated; (1) widely separated. Usually contiguous in Diptera (Schlein, 1970; Fabian *et al*., 2016) but widely separated in Pupipara (Massonat, 1909; Hennig, 1941; Bequaert, 1953: fig. 9A; Nußbaum, 1960: fig. 2; Schlein, 1970). Narrowly separated in Glossinidae.
25. Shape of metafurcasternum: (0) straight or only moderately curved; (1) s-shape; (2) z-shape. S-shape in *Melophagus* and z-shape in *Nycteribiidae*, correlated with increased flattening of the thorax (Schlein, 1970).
26. Paired metanotal process: (0) absent; (1) present. Present and projecting into the abdomen in Streblidae and Nycteribiidae (Schein, 1970). Possibly related with pupiparity according to Schlein (1970).

Legs

27. Position of coxae: (0) ventral; (1) dorsal. All coxae of *Melophagus* and Nycteribiidae are shifted onto the dorsal side of the body (Massonat, 1909: fig. 14; Nußbaum, 1960; Peterson & Wenzel, 1987: fig. 112.3).
28. Shape of procoxa: (0) not inflated; (1) inflated; (2) elongated and nearly cylindrical. Inflated and freely movable in different directions in *Crataerina* and members of Hippoboscidae (Bequaert, 1953: fig. 8a). According to Massonat (1909) the robustness ("robustesse") of the coxa increases with the degree of fixation to the host. More elongated and nearly cylindrical in *Basilia* (Nycteribiidae) (Nußbaum, 1960: fig. 2).
29. Mesocoxal ctenidium: (0) absent; (1) present. A thoracic ctenidium with a row of strongly developed, curved spines is formed as an elongation of the mesocoxa of Nycteribiidae (Schlein, 1970). This feature apparently is an autapomorphy of this family. It can be moved by a leg muscle (Schlein, 1970: fig. 23; Hennig, 1973) and is used to improve the anchorage in the fur of the bat.
30. Movability of mesotrochanter: (0) mainly between trochanter and coxa; (1) movably connected with coxa and femur. An increased movability between mesotrochanter and mesofemur in Pupipara is arguably linked with an increased fixation of the mesocoxa (Bequaert, 1953).
31. Shape of protarsomere 1: (0) not elongated and curved; (1) elongated and curved. Very distinctly elongated and curved in *Eucampsipoda hyrtli* and other nycteribiids examined.
32. Shape of protarsomeres 2-4: (0) longer than wide; (1) as long as wide or shorter. Tarsomeres 2-4 are distinctly shortened and compact in Pupipara (Massonat, 1909: fig. 22, pls. I, III, IV, VI, figs. 10, 24, 32, 48; Bequaert, 1953: fig. 12C, D; Maa & Peterson, 1987; Wenzel & Peterson, 1987).
33. Broad flattened heel of claws: (0) absent or heel weakly developed; (1) distinctly developed. Claws with a conspicuous, broadened and flattened base

or heel ("Krallengelenkhöcker") are characteristic for Pupipara (Massonat, 1909: fig. 21; Bequaert, 1953: figs. 12F, K).

34. Division of claws: (0) undivided claws; (1). Claws bifid (longitudinally split). Two longitudinal separate subunits of each claw are characteristic for ectoparasites of birds (Massonat, 1909: *Crataerina*, *Stenepteryx*) with the noteworthy exception of *Ornithoica*. Together with the curved claw-like claw tooth they form a tridentate structure (Bequaert, 1953: fig. 12A).
35. Pulvillus: (0) oval or widening distally and densely set with adhesive hairs (acantae without sockets); (1) cylindrical or distally narrowing, elongated and soft, with distinctly reduced vestiture of short hairs. Oval or distally widening and densely set with adhesive hairs in many groups of Diptera including *Glossina* (Bauchhenns, 1979; Gorb, 1998; Beutel & Gorb, 2001; Friedemann *et al*., 2014: fig. 8). Elongated and modified as soft structures in Pupipara (Bequaert, 1953; Nußbaum, 1960: fig. 18). Usually cylindrical but distally narrowing in some Streblidae (Zeve & Howell, 1963: fig. 38). Short adhesive hairs are present, but the vestiture appears sparse compared to other groups of Diptera (Bequaert, 1953; Nußbaum, 1960; Friedemann *et al*., 2014).

Wings and associated structures

36. Mesothoracic wings: (0) normally developed, permanent or shed after short flight; (1) partly reduced, shortened and lancet-shaped; (2) completely absent. Shortened, lancet-shaped and not suitable for active flight in *Crataerina* and *Stenepteryx* (Massonat, 1909). Also showing different degrees of reduction in various other representatives of Hippoboscoidea, for instance in most Streblidae (Wenzel & Peterson, 1987). Always absent in Nycteribiidae and also missing in *Melophagus*, but present in "volant individuals" of related genera (Bequaert, 1953). Also completely reduced in the non-related bee parasite *Braula* (Braulidae) (Massonat, 1909; Bequaert, 1953; Hennig, 1973). Normally developed in Glossinidae and the majority of the other hippoboscoid taxa including some species of Streblidae (Schlein, 1970: *Nycteribosca aluaudi*; Wenzel & Peterson, 1987).
37. Elongated arm of posterior notal wing process: (0) present and connected with mesonotum; (1) absent. Absent in Hippoboscidae and Streblidae (Schlein, 1970).
38. Club-shaped projection of the third axillary sclerite (calypteron): (0) absent; (1) present. A conspicuous club shaped projection of the third axillary sclerite is present in Streblidae, but absent in all other groups under consideration (Schlein, 1970).
39. Mesobasalar apodeme: (0) present; (1) partly or completely reduced. Present in *Drosophila* (Fabian *et al*., 2016: basalar apophysis) and *Musca* (Schlein, 1970). Distinctly reduced or absent in *Glossina* and Hippoboscidae, and also missing or vestigial in Streblidae and Nycteribiidae (Schlein, 1970).

Muscles

40. Origin of prothoracic dorso-ventral muscles: (0) at least some on pronotum; (1) all on mesonotum. The shift of all prothoracic dorso-ventral muscles onto the mesonotum (or mediotergal plate) is a characteristic feature of Hippoboscidae (Figs. 5C, D) and Nycteribiidae (Nußbaum, 1960: fig. 20, 21). The shifted origins are likely due to the limited space on the pronotum. Sufficient attachment areas for the leg muscles are available on the mesonotum. The control of the forelegs is important for the fixation and locomotion on the host.
41. M. pronoto–coxalis lateralis (Idvm18): (0) present; (1) absent. Present and strongly developed in *Crataerina*. Also present in other groups of Pupipara and in Muscidae (Schlein, 1970). Missing in *Glossina* and *Drosophila* (Schlein, 1970; Fabian *et al*., 2016).
42. M. procoxa-cervicalis (Ipcm1): (0) present; (1) absent. Absent in *Crataerina*, *Melophagus* and Streblidae. Present in other members of Hippoboscidae and in Nycteribiidae (Schlein, 1970).
43. M. mesonoto-trochanteralis (IIdvm7): (0) present; (1) absent. The mesotergal depressor of the mesotrochanter (TDT muscle, starter muscle) is absent in Glossinidae and Hippoboscidae but present in Streblidae and Nycteribiidae (Schlein, 1970; Hennig, 1973).
44. M. metanoto-sternalis (IIIdvm1): (0) present; (1) absent. Absent in *Drosophila*, *Crataerina* and *Melophagus* (Schlein, 1970; Fabian *et al*., 2016). Present in other Hippoboscidae including *Stenepteryx*, and also in Streblidae and Nycteribiidae (Schlein, 1970).

Additional characters not included in matrix: available information unspecific or insufficiently detailed, or gradually varying character states

1. Exposure of dorsal cervix and pronotum: (0) not covered by occipital region of head; (1) partly covered by occipital region of head; (2) completely covered by occipital region of head. Pronotum exposed in Glossinidae and other groups of Diptera (Hennig, 1973). Partly visible in *Ornithoica* and Hippoboscinae (Bequaert, 1953), and also in Streblidae (Hennig, 1973; Wenzel & Peterson, 1987: figs 113.1-2).
2. Notopleural suture: (0) distinct; (1) reduced. The notopleural suture is distinctly developed in *Ornithoica* but absent in Hippoboscinae (Hennig, 1973).
3. Mesopleural cleft. (0) not closed; (1) closed. Usually closed in flightless species such as *Melophagus* and Nycteribiidae (Bequaert, 1953).
4. Position of prothoracic spiracle: (0) laterally; (1) dorsolaterally; (2) dorsally. Shifted dorsad and fully exposed in *Crataerina* and other genera of Hippoboscidae, as for instance *Ornithoctona* (Bequaert, 1953: figs 8A, 10;

Schlein, 1970). Placed dorsolaterally and not visible from above in *Ornithoica* (Maa, 1966: fig. 1) and also in *Hippobosca equine* (Bequaert, 1953: fig. 1E). The position within the family varies considerably. It is also placed on the dorsal side in *Basilia* (Nycteribiidae) (Nußbaum, 1960: fig. 2), but not in Streblidae (Zewe & Howell, 1963: figs 4, 5), Glossinidae (Schlein, 1970) and other groups of Diptera (Hennig, 1973).

5. Size of prothoracic spiracle 2: (0) not enlarged; (1) distinctly enlarged. Distinctly enlarged in *Crataerina* and some other genera of Hippoboscidae and also in *Glossina* (Schlein, 1970: fig. 5). Moderately sized or small in *Ornithoica* (Bequaert, 1953: fig. 8A; Schlein, 1970: figs. 8, 12, 14), Streblidae (Schlein, 1970: figs 19, 21; Wenzel & Peterson, 1987: fig. 5) and Nycteribiidae (Nußbaum, 1960: fig. 2). The character varies strongly in Hippoboscidae and the character state polarity is ambivalent.
6. Metathoracic pleural suture: (0) vertical line; (1) bent with a sharp angle. Bent and forming a sharp angle in Hippoboscidae (Bequaert, 1953).
7. Mesofurcasternum: (0) one completed plate; (1) pair of plates. Divided into two plates in Hippoboscidae (Schlein, 1970). The condition in Streblidae and Nycteribiidae is unclear.

DISCUSSION

PHYLOGENETIC IMPLICATIONS AND CHARACTER EVOLUTION (FIG. 6)

The calyptrate superfamily Hippoboscoidea is characterized by specialized blood-feeding habits and adenotrophic vivipary (Massonat, 1909; Bequaert, 1953; Hennig, 1973). Within the group, Glossinidae have largely maintained a generalized thoracic configuration, differing only slightly from the brachyceran groundplan (Schlein, 1970; Hennig, 1973). In contrast, the other families (combined as Pupipara) often display a strikingly modified pattern (Bequart, 1953; Schlein, 1970). Hippoboscidae, Nycteribiidae and Streblidae are characterized by far-reaching character transformations, apparently often directly linked with specialized ectoparasitism on mammals and birds.

Derived thoracic features shared by Glossinidae and Pupipara are relatively scarce. A potential synapomorphy is the median separation of the procoxae [9: 1] (numbers in square brackets refer to characters and character states; see Fig. 6). However, the condition observed in *Glossina* (Bequaert, 1953: fig. 9B; Schlein, 1970) differs only slightly from what is found in other dipteran groups (Hennig, 1941: pls. 8, 9). Other potential synapomorphies are reduced conditions of both the mesepisternal ridge [16: 1] and mesobasalar apodeme [39: 1], and the ventral fusion of the metepimera [23: 1] (Schlein, 1970).

In contrast to Hippoboscoidea, Pupipara are supported by a multitude of unusual features, with a thoracic pattern very distinctly modified compared to a presumptive

groundplan of the superfamily. Conspicuous synapomorphies of the three families are the partial obliteration of the dorsal segmental borders [1: 1] and the distinct flattening of the entire thorax [2: 1], which is about half as high as wide in *Ornithoica*, but usually only one third in the other groups. In the typical case, the dorso-ventral compression of the thorax is linked with a flat or even concave mesopleura (Bequaert, 1953). Derived features of the prothorax are a distinct shortening of the pronotum [8: 1] and the increased size and movability of the procoxa [28: 1]. The mesothorax is apparently the most strongly modified segment. In contrast to Glossinidae and other groups of Diptera, it is strongly broadened [13: 1]. The mesocoxae are widely separated [17: 1], and the mesobasisternum is strongly extended [18: 1]. The movability between the mesotrochanters and the mesofemora is increased [30: 1]. The metacoxae are widely separated, largely immobilized and strongly increased in size [24: 1]. The proximal tarsomeres (at least 2-4) of all legs are shortened, broadened and flattened [32: 1]. A broad, heel-like claw tooth (Bequaert, 1953a: heel; fig. 12K) is present at the base of the strongly developed claws [33: 1]. In contrast to the typical brachyceran condition (Beutel & Gorb, 2001; Friedemann *et al.*, 2014: *Glossina*), the pulvilli are transformed into flexible cylindrical (or apically tapering) structures, with a vestiture of minute hairs (acanthae) [35: 1].

Bat flies, including Streblidae and Nycteribiidae, form a clade in our analyses as they do in Petersen *et al.* (2007). Potential thoracic synapomorphies of the two families are the loss of the first lateral cervical sclerite [6: 1], a largely reduced probasisternum [10: 1] and the presence of paired metanotal processes projecting into the abdomen [26: 1]. Another complex and unusual feature is a specific deformation of the anterior end of mesobasisternum [19: 1]: it is folded upwards and backwards, displacing the profurca on a second plane.

The non-monophyly of Streblidae (Peterson *et al.*, 2007) is not addressed here, as we included only one relatively unmodified terminal taxon with available detailed anatomical data (*Nycteribosca*). An unusual derived feature occurring in the family is a club-shaped projection of the third axillary sclerite [38: 1]. In contrast to the other two families, the body shape varies strongly in Streblidae, with some forms not compressed dorso-ventrally but laterally (Wenzel & Peterson, 1987).

Nycteribiidae are characterized by an entire series of character transformations, and most of them are linked with the complete reduction of the flight organs. The wings are completely reduced [36: 2], and the thoracic dorsum is largely membranized [3: 1]. The mesopleural cleft is closed, the mesofurca vestigial [20: 1], and the direct flight muscles are almost completely reduced (see EA1). The coxae are moved dorsad, similar to the condition in *Melophagus* [27: 1]. Protarsomere 1 is distinctly elongated and curved, at least in the species examined [31: 1]. The mesocoxal ctenidium, which provides additional anchorage in the fur of the bat, is a unique autapomorphy [29: 1]. Cephalic ctenidia have evolved independently within Streblidae, apparently with a similar function (Dick & Patterson, 2006).

It is noteworthy that our analysis did not confirm the monophyly of Hippoboscidae. This result might agree with a recent classification of Diptera (Pape & Thompson, 2018), which includes both Nycteribiidae and Streblidae into this family, replacing the original Pupipara. Interestingly, *Ornithoica*, a genus from Ornithoicinae (Hennig, 1973) or Ornithomyiinae, was placed as sistergroup of the entire remaining Pupipara, thus rendering the louse flies in the traditional sense indeed paraphyletic. An isolated position of the genus was already discussed by Hennig (1973), who placed the genus in a separated superfamily (Bequaert, 1953), and also by Maa (1966) who listed an entire series of plesiomorphies of different body regions. In our analysis, apomorphies of Pupipara excluding *Ornithoica* are the far-reaching obliteration of the dorsal segmentation [1: 2] (Bequaert, 1953: less indistinct in *Ornithoica*) and a higher degree of flattening of the thorax [2: 2] (reversal in some Streblidae). Thoracic plesiomorphies preserved in *Ornithoica* include weakly developed humeral calli [14: 0], the presence of a well-developed notopleural suture, the dorso-lateral placement of the prothoracic spiracle (also in some other Hippoboscidae), weak lateral thoracic grooves for receiving the profemora, functional wings [36: 0], extensive wing-cilia, a large alula, almost complete venation (compared with the other hippoboscid genera), slight dissimilarity in length and shape of the three pairs of legs, and one-toothed claws (Bequaert, 1953; Maa, 1966). Additional plesiomorphies are the completely separated basal antennomere, a short and simple frons, completely developed ocelli, a concealed interantennal area, comparatively well-developed tergal plates (both sexes), a relatively complete sclerotization of the abdominal sternites, and wide host and distributional ranges (Maa, 1966). *Ornithoica* was not included in the sampling of Petersen *et al.* (2007). However, the position of this genus definitely deserves increased attention.

The monophyly of Hippoboscidae excluding *Ornithoica* is not supported in our analysis. Not surprisingly, *Crataerina* is placed as sister taxon of *Stenepteryx*, ectoparasite of the house martin. Both taxa share similar streamlined connection of head and thorax [4: 1], multiple-teeth [34: 1] and a similar pattern of wing reduction [36: 1]. Nevertheless, in contrast to Petersen *et al.* (2007), the loss of the flight capacity may have occurred independently, as suggested by different muscular patterns in the thorax (see EA1).

ADAPTATIONS TO ECTOPARASITISM

Adaptations linked to the reproductive mode and larval development were previously described (Meier *et al.*, 1999; Petersen *et al.*, 2007). Transformations of external thoracic characters were discussed in Massonat (1909), Bequaert (1953), Schlein (1970), Maa & Peterson (1987) and other contributions.

Adults of Pupipara are characterized by a multitude of features more or less closely related to blood feeding, ectoparasitic habits and adenotrophic vivipary (Massonat, 1909; Bequaert, 1953; Maa & Peterson, 1987). Apart from a tendency towards

reduction of the flight organs and a compact unit formed by the head and anterior thorax, obvious adaptations are the flattening of the body (Wenzel & Peterson, 1987: exception in some Streblidae) and the rigid connection of the thoracic segments, with the segmental borders obliterated on the dorsal side (Massonat, 1909). Combined with a resilient, "leathery cuticle" (Bequaert, 1953), this renders the flies very resistant against mechanical damage resulting from defensive movements of the hosts. What requires further investigation, however, is how the ectoparasites prevent injury of internal organs when their body is strongly squeezed.

A side effect of the compaction of the thorax is a posterior shift of dorso-ventral muscles of the prothorax. Their mesonotal origin in Pupipara is an unusual feature probably also linked with the reduced size of the pronotum. A feature characteristic of the family Hippoboscidae is the dorsal shift (and large size) of the prothoracic spiracles (Bequaert, 1953; Schlein, 1970). This is likely correlated with the widening and flattening of the mesothorax. It is possible that the exposed position improves the ventilation of the middle thoracic region of louse flies. However, this is not a general feature of Pupipara. Moderately sized prothoracic spiracles are present in *Ornithoica* and also other members of the group. Spiracles reduced in size may be correlated with a strongly reduced flight apparatus. A flightless lifestyle requires less energy and therefore a lower supply of oxygen.

The legs are strongly developed compared with the other groups of Diptera (Bonhag, 1949; Smart, 1959; Ulrich, 1971, 1984; Owen, 1977; Fabian *et al.*, 2016). They are affected in various ways by ectoparasitic habits. Dufour (1845) observed that hippoboscids are able to walk efficiently backwards and laterally without raising their flattened body from the substrate. This ability is presumably linked with the rather freely suspended procoxae, and meso- and metatrochanters with high degrees of freedom. The modifications of the legs apparently enhance the attachment and movability on the hairy or plumed integument of the warm-blooded vertebrate hosts. A conspicuous feature, which is not found in other groups of flies, is the wide separation of the coxal insertions. This likely improves the grasp on the hosts. It reaches a culminating point in the sheep ked *Melophagus* and in nycteribiid bat flies (Massonat, 1909; Bequaert, 1953): the coxae of these advanced ectoparasites are moved onto the dorsal side of the body, a highly unusual feature in insects. The laterally shifted coxal insertions apparently correlate with the dorso-ventral flattening of the body. Due to this modified coxal arrangement, it is also possible to shift the origin of coxal and trochanteral muscles. *Melophagus*, which lives submerged in the wool of its ovine host (Massonat, 1909: "plongé dans la laine de son hôte"), shows an exceptionally high degree of specialization compared to other hippoboscids. The thorax is shortened and very compact. The mesonotal sutures are largely obliterated and the flight organs completely reduced (Bequaert, 1953). According to Bequaert (1953), the sheep ked also reaches a maximum degree of mechanical resilience.

The articulation of the coxae of Pupipara is generally modified compared to other flies. The enlarged procoxae are very movable, whereas the mesocoxae and the

enlarged metacoxae are largely or completely immobilized. This lack of coxal movement is compensated for by an increased movability in the trochanteral-femoral joint, at least in the case of the mid legs (Schlein, 1970). This enhanced rotation may be beneficial during the attachment process, because it supports the adjustment of the attachment angle of the pretarsus. The tarsi are generally shortened and broadened. The protarsal attachment structures differ indistinctly from the typical brachyceran pattern (Bauchhenss, 1979; Friedemann *et al.*, 2014), likely due to the necessity to cling to moveable hosts with feathers or fur. The pulvilli are strongly modified in *C. pallida* and other hippoboscids, flexible, cylindrical and covered with a vestiture of specialized minute tenant hairs. Additionally, a slender empodium is present, partly glabrous but partly covered with very short setae. The strongly developed claws play a major role in attachment and locomotion on the host body surface. They are bifid (longitudinally cleft) in ectoparasites of birds, with the noteworthy exception of the genus *Ornithoica*. Bifid claws combined with an elongated and curved claw tooth (Friedemann et al., 2014) may improve the grasp and movability in the plumage.

Claws showing edges with multiple-teeth occur in spiders (Aranae), and ensure an efficient grasp on filamentous structures of their safety threads and orb webs (Gorb & Barth, 1994). This is structurally (and probably functionally) very different from the claw apparatus of bird ectoparasites in Hippoboscidae, where the bifid claws are supported by a curved claw tooth, thus forming a trifid functional unit but without a serrate (or multi-toothed) edge.

FLIGHTLESSNESS

Despite profound modifications linked with anatomical dipterism, the pterothoracic flight apparatus of true flies is a highly complex functional unit like in other groups of Pterygota (Brodsky, 1994; Deora, Gundiah & Sane, 2017). Boettiger & Furshpan (1952) and Boettiger (1957) observed and described the dipteran flight mechanism for the first time in detail using *Sarcophaga bullata* Parker, 1916 (Sarcophagidae). After that, Pringle (1957) proposed a hypothetical 3D model, which was later evaluated and demonstrated with a mechanical model designed by Pfau (2008). A synchronous wing receptor was described by Miyan & Ewing (1984), wing base articulations and the muscular system by Miyan & Ewing (1985), direct flight muscular modulation by Tu & Dickinson (1994, 1996), and the skeletomuscular system of wingless mutants by Fabian *et al.* (2016). The haltere function as mechanosensory control organ was treated in Chapman (1982), wingbeat-synchronous feedback in Fayyazuddin & Dickinson (1999) and passive mechanical coordination by Deora, Singh & Sane (2015). Recently, Deora *et al.* (2017) summarized various structures of the dipteran flight apparatus in detail. The evolutionary pattern of wing reduction in Hippoboscoidea was addressed in a phylogenetic study based on molecular data (Petersen *et al.*, 2007).

Well-developed flight organs are doubtlessly part of the groundplan of Hippoboscoidea and Pupipara. However, as outlined in Massonat (1909), Bequaert (1953), Schlein (1970) and more recent contributions (Maa & Peterson, 1987; Wenzel & Peterson, 1987), various patterns of reduction occur within the group. In Hippoboscidae wings can be partly (e.g. *Craterina*, *Stenepteryx*) or completely reduced, they can be shed shortly after emergence or after reaching a suitable host (e.g. some species of *Hippobosca*), or represented by a solid subcylindrical knob, obviously unsuitable for flight (Maa & Peterson, 1987). A strong tendency towards flightlessness characterizes the Streblidae (Schlein, 1970; Wenzel & Peterson, 1987) and the development culminates in Nycteribiidae. All known species of this family have lost their wings. The flight muscles are almost completely reduced (see Electronic Appendix 1). The thoracic dorsum is largely membranous and the entire body strongly flattened (Nußbaum, 1960; Wenzel & Peterson, 1987).

The flight capacity of *C. pallida* is minimal if not lost completely. In laboratory experiments, the flies were only able to perform small jumps and glide for very short distances in the air (Eichler, 1939; Büttiker, 1944). Like species of other groups of pterygote insects, *C. pallida* and other hippoboscids are subject to a trade-off between obvious advantages of flight (e.g. efficient escape mechanism, dispersal) and adaptations to a specific ecological condition of a single host, in the case of Pupipara ectoparasitism (Roff, 1990; Wagner & Liebherr, 1992). Important subcomponents of the flight apparatus of *C. pallida* are reduced or non-functional. The mesothoracic wing base sclerites are indistinct and partly merged with the membranous notopleural region, similar to a condition observed in flightless females of the Japanese winter moth *Nyssiodes lefuarius* (Erschoff, 1872) (Liu *et al.*, 2017). In pterygote insects with a functional flight apparatus, they form an efficient articulation with a click mechanism to transmit force from the thoracic exoskeleton to the wings (Brodsky, 1994; Pfau, 2008; Deora *et al.*, 2017). This mechanism is apparently not possible with the vestigial articulation of *C. pallida*. Another strongly modified sclerotized structure is the mesothoracic pleural wing process, which forms the wing fulcrum on the ventral side of the wing base in insects capable of flight (Brodsky, 1994). In dipterans with a normally developed flight apparatus, the mesopleural wing process is a prominent elongate rod-shaped structure (Bonhag, 1949; Smart, 1959; Schlein, 1970; Ulrich, 1971, 1984). In contrast, it is only preserved as a tiny process on the dorsal mesepimeral margin in *C. pallida*.

The dorsal longitudinal muscle IIdlm1 of dipterans is usually divided into several large bundles connected with the extensive anterior area of the mesonotum (Maki, 1938; Bonhag, 1949; Smart, 1959; Christophers, 1960; Mickoleit, 1962; Ulrich, 1971, 1984; Owen, 1977; Fabian *et al.*, 2016). This is also the case in hippoboscid species with preserved flight capacity (Schlein, 1970). In contrast, in *C. pallida* the origin of this indirect flight muscle is shifted backwards to the posterior mesonotal region. The muscle is distinctly shortened compared with the usual condition in dipterans and composed of a narrow single bundle. From its size and position it is clearly unsuitable

for providing the initial contraction of the segment, which is necessary for wing movements (Brodsky, 1994; Pfau, 2008; Deora *et al.*, 2017). The absence of the dorso-ventral muscles II/IIIdvm1, 4 and 5 is obviously related to flightlessness. Especially IIdvm1 is reduced in all studied flightless polyneopteran and holometabolan species (Friedrich & Beutel, 2010b; Wipfler *et al.*, 2014; Liu *et al.*, 2017). As indirect wing levators (Brodsky, 1994; Pfau, 2008; Deora *et al.*, 2017), these three muscles are almost generally present in dipterans with a functional flight apparatus (see EA1). Meanwhile, with the loss of several flight muscles, more space in the thoracic lumen becomes available for enlarged extrinsic leg muscles, including the dorsoventral muscles Idvm15, 18 and 19 and the sterno-coxal muscles Iscm1–4, 6, IIscm1–3, 6 and IIIscm1–3, 6 (Fig. 5). It is conceivable that this enhances the capacity of the legs to attach on the hosts integument.

The mesothoracic direct flight muscles are scarcely affected by the loss of the flight capacity. Only the tergo-pleural muscle IItpm2 and sterno-pleural muscle IIspm1 are absent, compared with most other dipterans capable of flight (see EA1). The tergo-pleural muscles were defined as synchronous steering muscles that control higher-order wing kinematic patterns, such as a gear shift mechanism (Pfau, 2008; Deora *et al.*, 2017). The sterno-pleural muscle IIspm1 is absent in most studied flightless insects (Friedrich & Beutel, 2010b; Wipfler *et al.*, 2014). It probably functions primarily as an indirect wing levator (Friedrich & Beutel, 2008), but may have secondary functions like adduction and pronation during wing strokes (Brodsky, 1994). Although the dipteran metathorax is distinctly reduced, the halteres play an important role as a gyroscope mechanism during flight (Deora *et al.*, 2017). Some muscles connected to these structures support flight maneuvers (Fabian *et al.*, 2016). In *C. pallida*, the absence of the tergo-pleural muscle IIItpm11 is likely linked with flightlessness. This muscle is present in species of Hippoboscidae capable of flight (see EA1).

Some flight related muscles preserved in *C. pallida* apparently have more than one function (Brodsky, 1994). Several tergo-pleural muscles fulfill the additional function of stabilizing the dorsolateral region (Kozlov, 1986; Brodsky, 1994). The sterno-pleural muscle IIspm2 provides a part of the elastic energy required for the click mechanism at the wing base, and it is also involved in gear shifting during flight (Pfau, 2008). It also stabilizes the relative positions of mesopleuron and mesofurca, which form an integrated endoskeletal complex (Kozlov, 1986).

Some partly or completely preserved structures of the flight apparatus of *C. pallida* are potential evolutionary relicts (Liu *et al.*, 2017). This applies to the indistinct but present wing base sclerites, a mesopleural wing process which is only partly reduced, and some retained pterothoracic muscles. Conversely, some flight related muscles can be absent in dipterans with a functional flight apparatus, such as for instance the dorso-ventral indirect wing levator muscle IIdvm1, which is lacking in *Drosophila*, or the dorso-ventral muscle IIdvm7, which is missing in species of Hippoboscidae capable of flight (see EA1). Their absence is likely compensated by other elements of

the skeletomuscular system. This phenomenon was observed in species of Orthoptera and Blattodea (Brodsky, 1994) and recently also in whirligig beetles with a strongly simplified but functional flight apparatus (Liu, Wipfler & Beutel, 2018).

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Table 1: Character matrix used for the cladistics analysis of Hippoboscoidea

Taxon	Character states								
Drosophila	00000	0?000	00000	00000	00000	00000	00000	00000	1001
Musca	00000	00000	00000	00000	01000	00000	00000	00000	0000
Glossina	00001	0?010	10000	10000	01100	00000	00000	00010	1010
Ornithoica	1100?	0?110	??10(01)	111??	?011?	?0101	01101	0101?	????
Hippobosca	22000	00110	10101	11100	00110	00101	01101	01011	1010
Melophagus	2200?	01110	?0101	01100	00111	01101	01101	21011	1111
Crataerina	22010	01110	10111	11100	00110	00101	01111	11011	1111
Stenepteryx	2201?	?1110	?0111	11100	00110	00101	01111	11011	1010
Nycteribosca	22001	11111	00101	11110	02110	10101	01101	01110	1100
Eucampsipoda	22101	1?111	0110-	11111	1-112	11211	11101	2-010	1000

ELECTRONIC APPENDIX

Appendix 1: Diptera muscular homology chart. Muscle present is represented by "+" in green, absent by "-" in pink, uncertain by "?" or "/" in yellow.

ACKNOWLEDGEMENTS

We thank to Arndt Wellbrock, Klaudia Witte (University of Siegen, Germany) and Nils Kreuter (Kiel University, Germany) for providing specimens of *C. pallida*. We also thank Dr. M. Jäch and P. Sehnal, curators of the Naturhistorisches Museum Wien, for providing the opportunity to examine other species of Hippoboscoidea. This project was financially supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 642241.

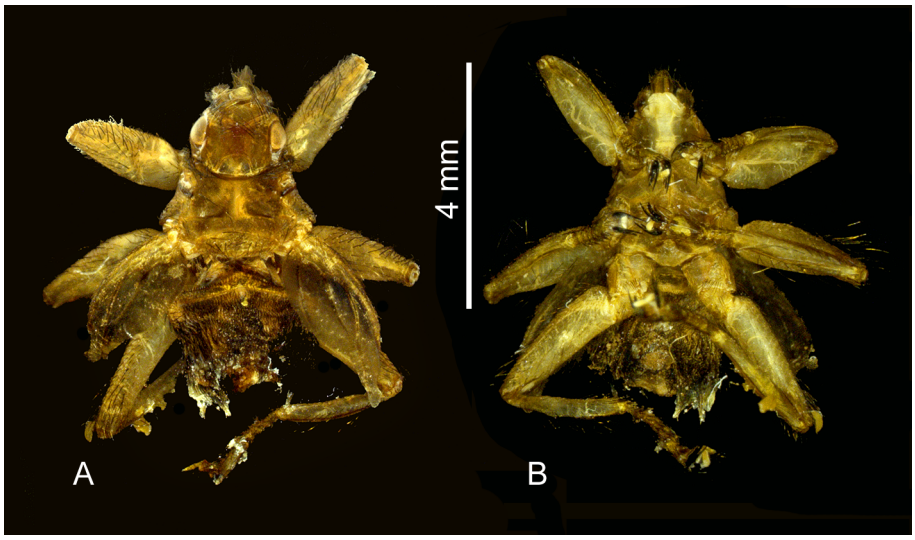

Fig. 1. *Crataerina pallida*, digital photographs, habitus. (a) dorsal view; (b) ventral view.

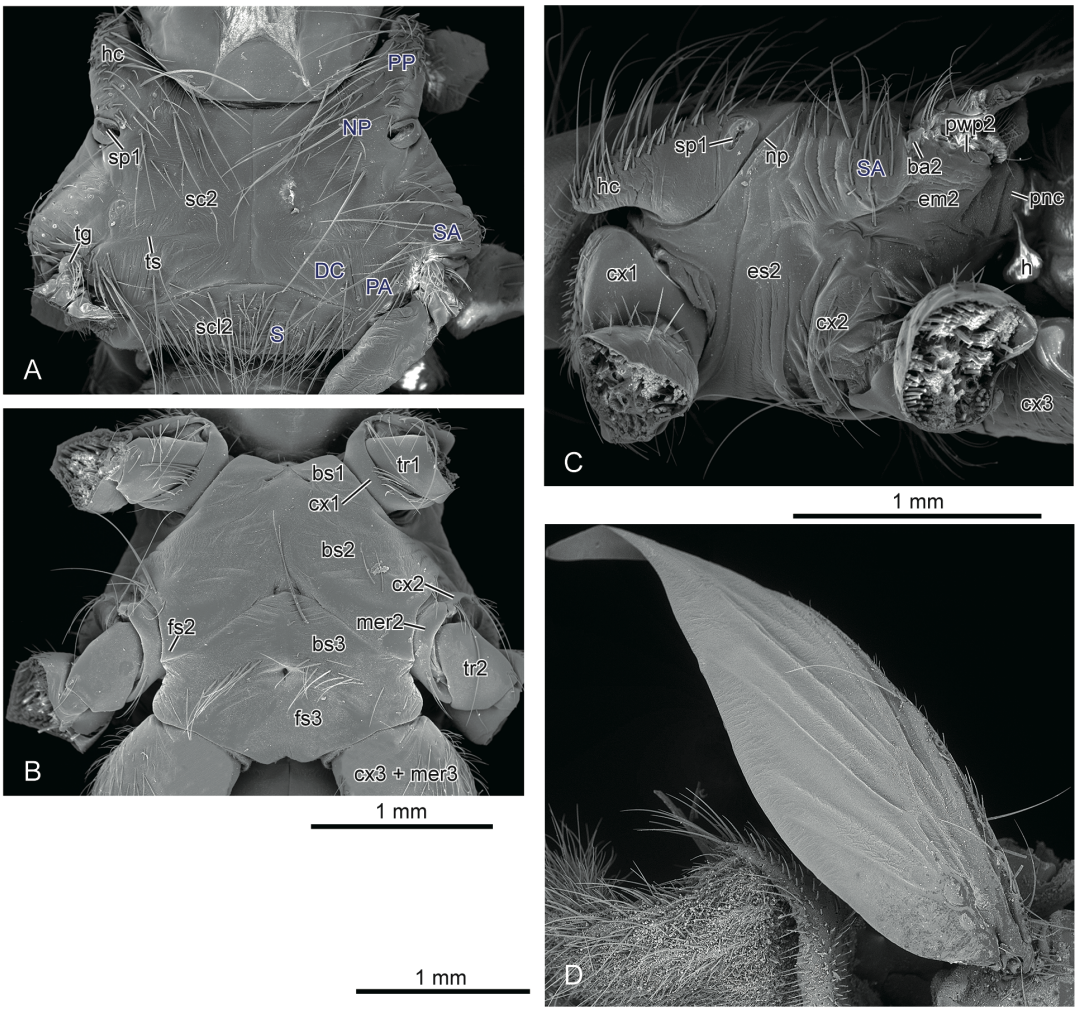

Fig. 2. *Crataerina pallida*, SEM micrographs, thorax. (a) dorsal view; (b) ventral view; (c) lateral view; (d) wing.

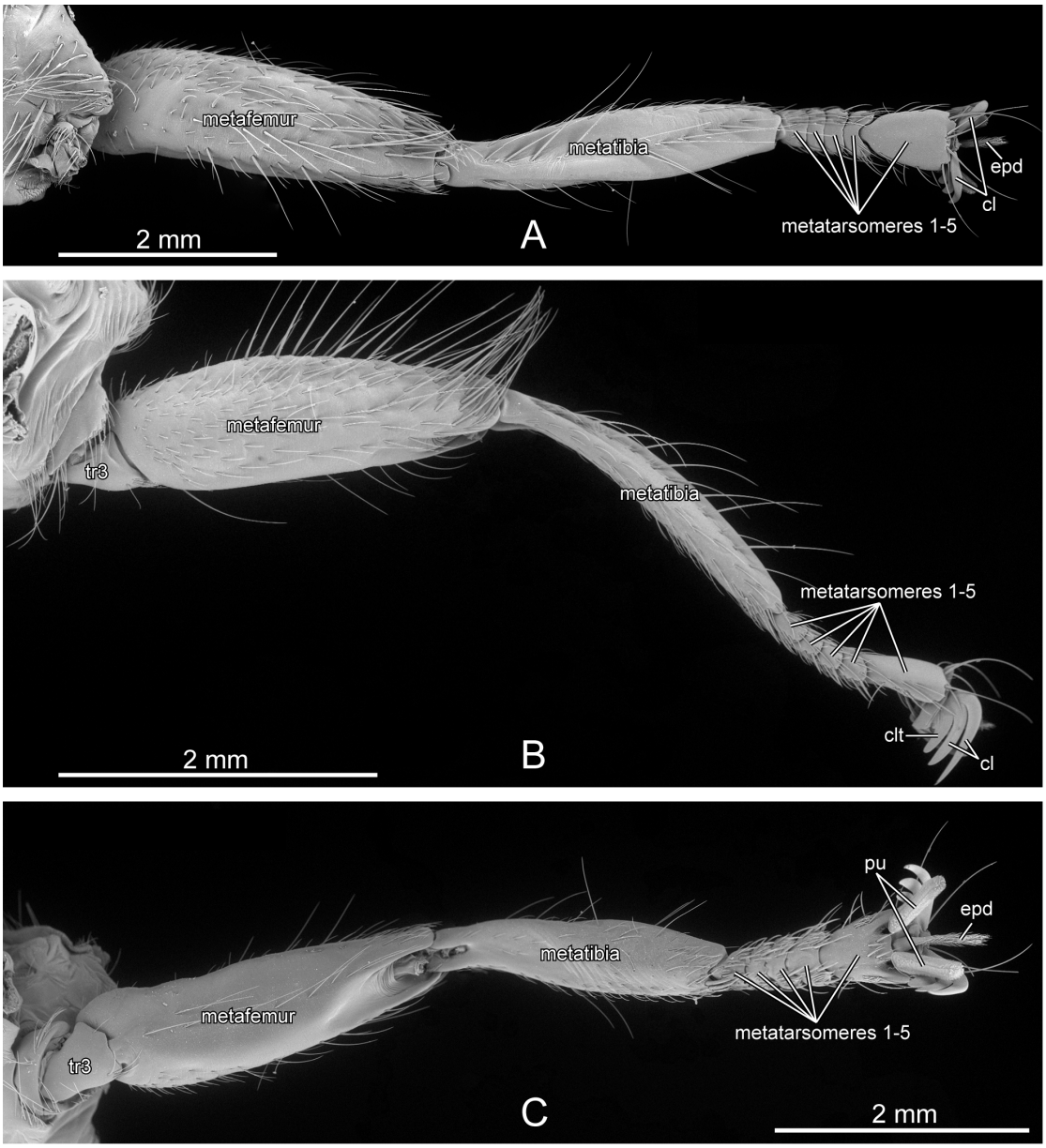

Fig. 3. *Crataerina pallida*, SEM micrographs, hind legs. (a) dorsal view; (b) lateral view; (c) ventral view.

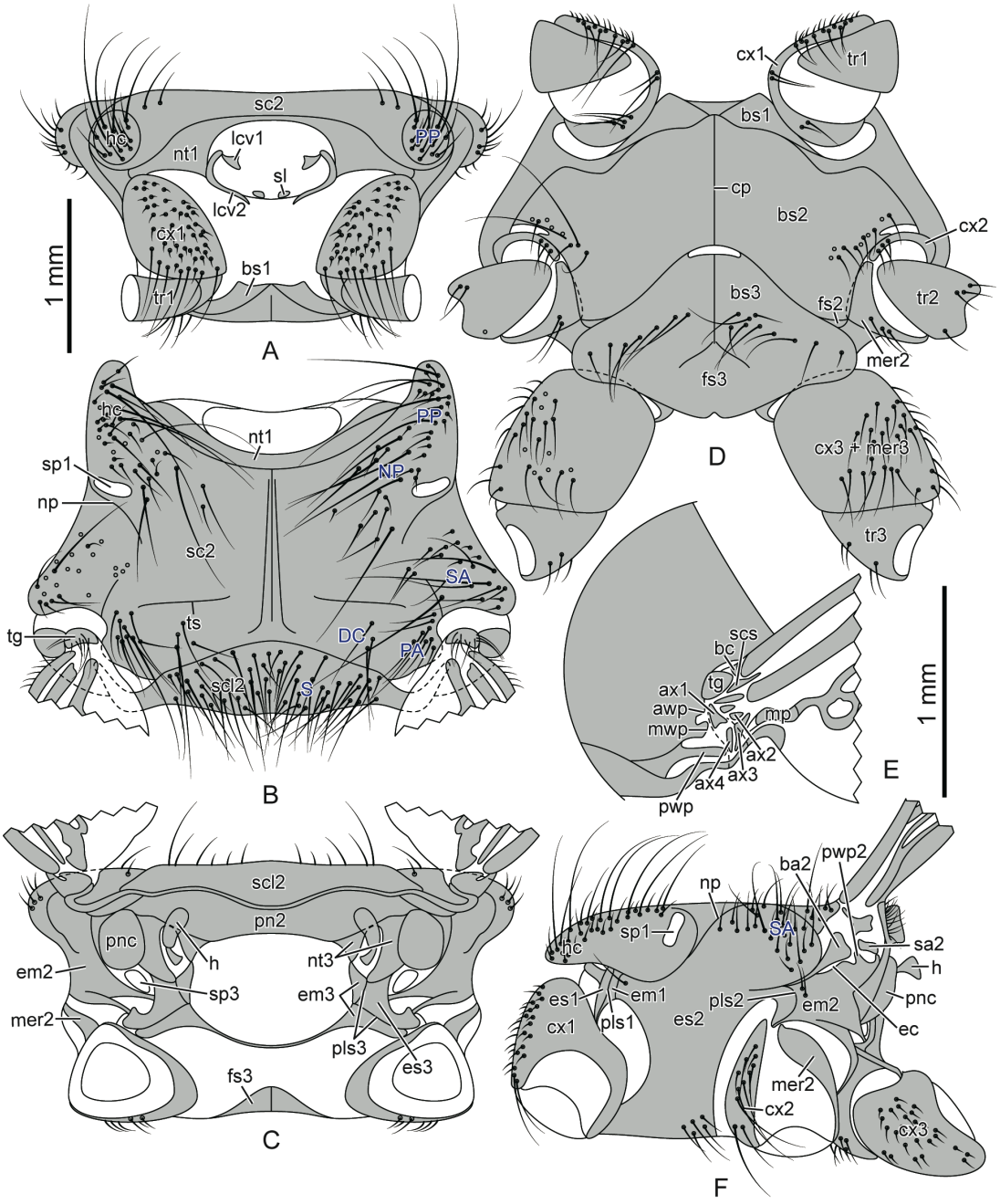

Fig. 4. *Crataerina pallida*, line drawing, thoracic skeleton. (a) front view; (b) dorsal view; (c) hind view; (d) ventral view; (e) wing base; (f) lateral view. Scale bars: upper 1 mm for (a–d, f); lower 1 mm for (e).

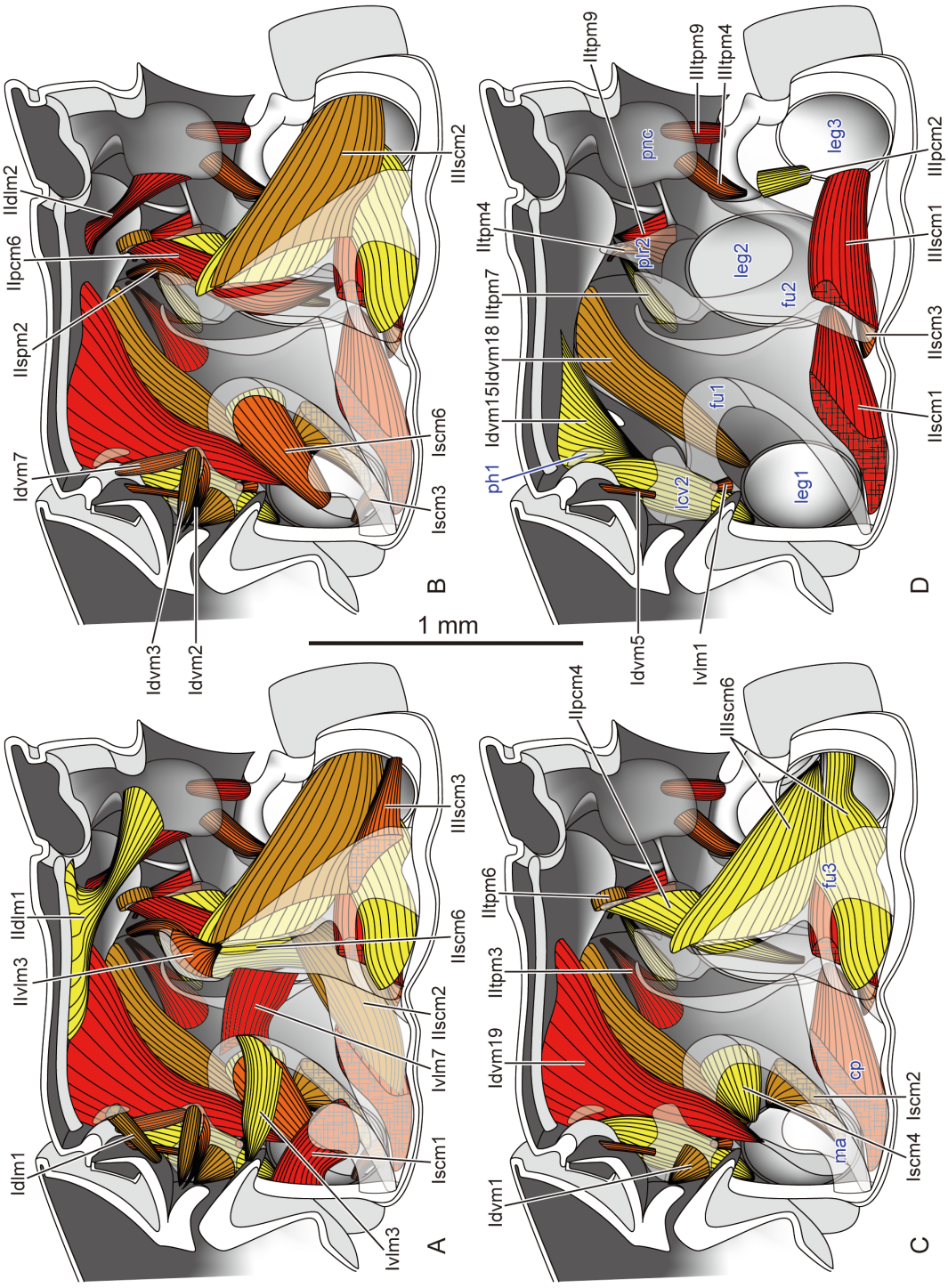

Fig. 5. *Crataerina pallida*, line drawing, thoracic endoskeleton and muscles. Lateral view. Skeletal structures labeled in blue, muscles in black. The muscles are removed layer by layer from A to D.

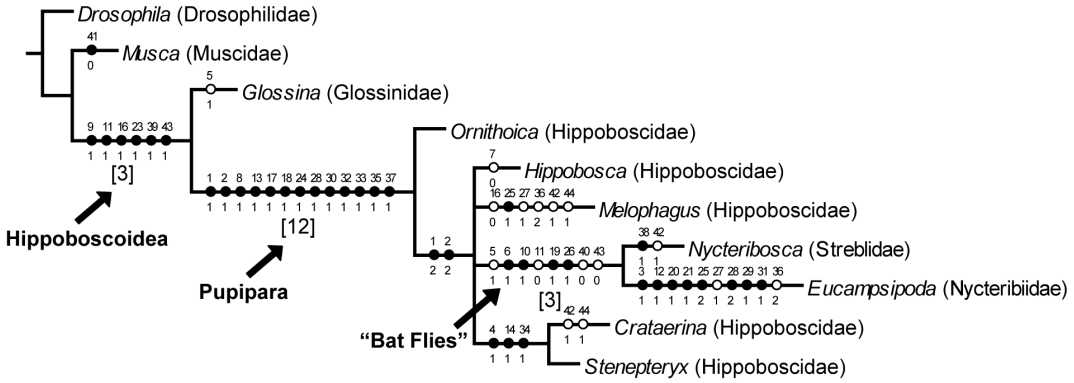

Fig. 6. Strict consensus tree of 8 equally parsimonious trees (65 steps, CI = 76, RI = 78) of combined analysis of 44 thoracic morphological data of Hippoboscoidea (NONA, Ratchet search, 1000 iterations). Apomorphies mapped on the tree. The Bremer support values more than 1 are labeled under the corresponding branches.

3.3.2. Published Version

Zoological Journal of the Linnean Society, 2018, **XX**, 1–21. With 6 figures.

The thoracic anatomy of the swift lousefly *Crataerina pallida* (Diptera)—functional implications and character evolution in Hippoboscoidea

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Received 10 December 2017; revised 10 April 2018; accepted for publication 18 April 2018

The thoracic skeletomuscular system of the swift lousefly *Crataerina pallida* (Diptera: Hippoboscidae) is documented with scanning electron microscopy, micro-computed tomography and three-dimensional reconstruction. The morphological results are discussed with respect to ectoparasitism and flightlessness. The evolution of thoracic characters in Hippoboscoidea is reconstructed based on a parsimony analysis of 44 thoracic characters. The results confirm the monophyly of Hippoboscoidea, Pupipara (Hippoboscidae + Streblidae + Nycteribiidae) and the bat flies (Streblidae + Nycteribiidae). The monophyletic origin of Hippoboscidae is challenged with respect to the genus *Ornithoica*, which displays several plesiomorphic features compared with conditions shared by the remaining Pupipara. The thorax of the species in the three families is distinctly affected by the ectoparasitic lifestyle. The dorsal segmental borders are obliterated, and the thorax is usually strongly flattened. The legs are adapted to cling to the host and to move efficiently in the fur or plumage. A heel-like claw tooth supports large claws. Different patterns of reduction of the flight apparatus occur in the group. Even though *Crataerina* and *Stenepteryx* are close relatives and similar in their general thoracic configuration, differences in the muscular patterning suggest independent losses of the capacity for flight.

ADDITIONAL KEYWORDS: ectoparasite – evolution – flightlessness – Hippoboscoidea – thoracic morphology.

INTRODUCTION

Hippoboscidae, commonly known as louse flies or keds, are an exceptional group within the highly diverse Diptera. All species are parasitic and feed exclusively on fresh blood of homoiotherm vertebrates (Massonat, 1909; Bequaert, 1953; Maa & Peterson, 1987). This kind of specialized nutrition is rather rare among insects, but can be found in different groups of Diptera, including, for instance, 'nematoceran' biting flies and black flies, but also tse-tse flies (Glossinidae) and bat flies (Nycteribiidae and Streblidae), which are close relatives of Hippoboscidae (Nycteribiidae and Streblidae were included in Hippoboscidae in alternative classifications; Griffiths, 1972; Pape & Thompson, 2018). Hippoboscidae combined with bat flies and tse-tse flies form the Hippoboscoidea. With the possible exception of Streblidae (Petersen *et al.*, 2007), monophyly is widely accepted for all family-level taxa and the superfamily as a whole (Hennig, 1973; McAlpine, 1989).

All Hippoboscoidea are adenotrophic viviparous (Meier, Kotrba & Ferrar, 1999). It has been postulated that high temperatures in combination with sufficient nutrition, particularly haematophagy, are responsible for this kind of reproductive strategy (Roubaud, 1909; Senior-White, Aubertin & Smart, 1940). However, it is difficult to confirm this hypothesis because other insects living in similar conditions are not viviparous, and viviparity is not strictly associated with haematophagy (Meier *et al.*, 1999). In any case, hippoboscids apparently benefit from this reproductive adaptation; it results in a better protection of offspring from predation or parasitism, shortens the larval development

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and enables synchronization of hatching according to the presence of suitable hosts.

Shelter and forage, which are crucial for non-parasitic flies, are provided by the host in the life cycle of hippoboscids. However, reliable anchorage and efficient locomotion on the host become critical issues, especially for species with partly or completely reduced flight organs. The ectoparasitism requires far-reaching structural and physiological adaptations; for instance, a flattened body and legs with specialized attachment devices (Kemper, 1951). Adaptive specialization of different body parts has probably led to an obligate dependence on a single host species in many cases (e.g. Bequaert, 1953).

A striking feature of the group are the various modifications of wings across hippoboscoid taxa. The reduced wings are probably correlated with an increased mobility on the host and are frequently observed among ectoparasites (Andersen, 1997). The horse ked *Hippobosca equina* Linnaeus, 1758 has retained functional wings and is not restricted to a single host species. It also sucks blood from deer, dogs and humans (Hutson, 1984). In contrast, the deer fly *Lipoptena cervi* (Linnaeus, 1758), a poor flyer, sheds the wings after attaching to a suitable host (Hutson, 1984). Its host spectrum is restricted to deer and close relatives. The sheep ked *Melophagus ovinus* (Linnaeus, 1758) is entirely wingless, strongly associated with the domestic sheep, and spends its entire life cycle on this host (Hutson, 1984).

The swift louse fly *Crataerina pallida* (Olivier in Latreille, 1812), which is the focus of our study, displays an intermediate condition with regard to wing modification. The body is shortened, and its reduced forewings are unsuitable for active flight (Hutson, 1984). Possible functions of the partly reduced wings are still under debate. Eichler (1939) suggested that they are used to perform small jumps, and Büttiker (1944) allegedly observed that they are used to glide for short distances in the air. Walker & Rotherham (2010) suggested that the wings might provide an additional anchorage, thus improving attachment to the host. However, this interpretation also requires confirmation. Thus, the real function of the shortened wings still remains elusive.

Crataerina pallida is an extremely agile, monoxenous avian ectoparasite of the common swift. Its success strongly depends on a permanent reliable contact with the host. The resulting structural and physiological adaptations make it a promising model for investigating evolutionary changes linked to parasitism. The common swift is a migrating bird with a high nest fidelity (Weitnauer, 1947; Lack & Lack, 1951) and is present in Europe only from May to September. The life cycle of *C. pallida* is adjusted to this seasonal appearance of the host. It places its fourth-instar larvae, which pupate immediately on emergence from the female, directly in the swift's nest to overwinter (Bequaert, 1953). The hatching of the pupae is temperature sensitive and synchronized with the swift's return (Popov, 1965; Walker & Rotherham, 2010). Although *C. pallida* is strictly hematophagous and can remove up to 5% of the host's blood volume with a single bite, no detrimental effects on the host were found (Hutson, 1981; Lee & Clayton, 1995; Tompkins, Jones & Clayton, 1996; Walker & Rotherham, 2010), suggesting a reduction in virulence as a direct adaptation.

Apart from physiological and developmental adaptations (Meier *et al.*, 1999), structural modifications, especially in the thoracic segments, are apparently important for the success of this species. Swifts rely on their nests for only short periods, especially when breeding and feeding the offspring. Consequently, *C. pallida* must attach to the host and move across it efficiently both in the nest and during flight (Lack & Lack, 1951). Gustafson *et al.* (1977) and Henningsson *et al.* (2009) recorded that the swift can reach altitudes > 3500 m and velocities distinctly exceeding 40 km/h. Consequently, reliable attachment of the ectoparasite in these conditions is essential (Kemper, 1951).

Despite the various fascinating aspects of the biology and morphology of *C. pallida*, external and internal structures of the thorax are insufficiently known (Massonat, 1909). Here, a detailed description of the thoracic skeletomuscular system is provided using an array of modern techniques. The morphological results are discussed with respect to functional adaptations to the specialized lifestyle, with a special focus on wing reduction in *C. pallida* and related taxa. The character evolution in Hippoboscoidea is reconstructed based on a matrix of thoracic characters, which is analysed using parsimony. Finally, a table homologizing thoracic muscles of different representatives of Diptera is presented.

MATERIAL AND METHODS

One specimen of *C. pallida* was manually dissected in 70% ethanol under a Zeiss Stemi SV 11 with an additional Euromex Illuminator EK-1 lighting system (Carl Zeiss MicroImaging GmbH, Oberkochen, Germany). The line drawings were executed with a pencil under the microscope, scanned and finished with Adobe Illustrator CC (Adobe Systems, San Jose, CA, USA). Sclerites and body margins were drawn with full lines, margins below other sclerites and folds at the wing base with dotted lines. Wings and legs were

omitted, except basal elements, i.e. coxae, trochanters and wing base sclerites.

For micro-computed tomography (µ-CT) analysis, the samples were dehydrated in an ascending ethanol series, critical point dried with a Quorum E3000 (Quorum Technologies Ltd, Laughton, UK) and scanned using a Skyscan 1172 (Bruker micro-CT, Kontich, Belgium) desktop µ-CT. The scans were performed with 40 kV voltage, 250 µA current, 720 ms exposure and a rotation of 360° in steps of 0.25°. Images were obtained at a resolution of 2.2 µm per voxel. The µ-CT data were reconstructed with FEI Amira 6.0 (FEI, Mérignac, France). Segmented structures were exported as stacks of TIFF files into Volume Graphics VGStudiomax 2.0 (Volume Graphics, Heidelberg, Germany), which was used for volume rendering and filming. The quality of the µ-CT result was not sufficient for a clear visualization with a three-dimensional reconstruction. Therefore, the final skeletomuscular figures were drawn with Adobe Illustrator CC (Adobe Systems) based on the reconstructions and direct observation using the dissected specimens.

For scanning electron microscopic analysis, the samples were dehydrated in an ascending ethanol series, critical point dried with a Quorum E3000 (Quorum Technologies Ltd) and sputter-coated with gold–palladium (10 nm thickness; Leica Bal-TEC SCD500) (Leica Microsystems GmbH, Wetzlar, Germany). Afterwards, the samples were mounted on a rotatable sample holder (Pohl, 2010) and examined using a Hitachi TM3000 (Hitachi High-Technologies Corp., Tokyo, Japan) scanning electron microscope at an accelerating voltage of 15 kV.

The terminology for the thoracic skeleton follows that of Schlein (1970) and Friedrich & Beutel (2010a), for the wing base elements Fabian, Schneeberg & Beutel (2016), for distal leg structures Friedemann, Schneeberg & Beutel (2014) and for the chaetotaxy Maa & Peterson (1987) and Fabian *et al.* (2016). The dipteran thoracic muscle names used by Maki (1938), Bonhag (1949), Smart (1959), Christophers (1960), Nußbaum (1960), Mickoleit (1962), Schlein (1970), Ulrich (1971, 1984), Owen (1977) and Fabian *et al.* (2016) were homologized with the generalized neopteran muscular nomenclature of Friedrich & Beutel (2008). The muscle names of Friedrich & Beutel (2008) were also used for the description of the thorax of *C. pallida*.

Some additional dried specimens at Naturhistorisches Museum Wien were examined, which include the following: Glossinidae: *Glossina* spp.; Hippoboscidae: *Ornithoica pusilla* (Schiner, 1868), *Ornithomyiia avicularia* (Linnaeus, 1758), *Lipoptena cervi* (Linnaeus, 1758) and *Melophagus ovinus* (Linnaeus, 1758); Streblidae: *Nycteribosca kollari* Frauenfeld, 1855; Nycteribiidae: *Eucampsopoda hyrtli* Kolenati, 1856, *Nycteribia allotopa* (Speiser, 1901), *Cyclopodia horsfieldi* de Meijere, 1899, and *Stylidia hermanni* Leach, 1816.

Forty-four thoracic morphological characters were coded and entered in a data matrix for eight taxa and two outgroups (Table 1). The parsimony analysis was carried out with WinClada 1.00.08 and NONA 2.0 software (Goloboff, 1999; Nixon, 1999–2002) and TNT 1.1 (Goloboff, Farris & Nixon, 2008). Bremer support values (Bremer, 1994) were calculated with NONA 2.0.

Abbreviations

a/m/pwp, anterior/median/posterior notal wing process; ax1/2/3/4, first/second/third/fourth axillary sclerite; ba2, mesobasalare; bc, basicosta; bs1/2/3, pro/meso/metabasisternum; cl, claw; cp, cryptosternum; cx1/2/3, pro/meso/metacoxa; clt, claw tooth; DC, postsutural setae of dorsal central region; ec, mesepisternal cleft; em1/2/3, pro/mes/metepimeron; epd, empodium; es1/2/3, pro/mes/metepisternum; fu1/2/3, pro/meso/metafurca; fs2/3, meso/metafurcasternum; h, haltere; hc, humeral callus; I, insertion; lcv1/2, first/second lateral cervical sclerite; leg1/2/3, fore-/mid-/hindleg;

Table 1. Character matrix used for the analysis of cladistics of Hippoboscoidea

Taxon	Character states								
Drosophila	00000	0?000	00000	00000	00000	00000	00000	00000	1001
Musca	00000	00000	00000	00000	01000	00000	00000	00000	0000
Glossina	00001	0?010	10000	10000	01100	00000	00000	00010	1010
Ornithoica	1100?	0?110	??10(01)	111??	?011?	?0101	01101	0101?	????
Hippobosca	22000	00110	10101	11100	00110	00101	01101	01011	1010
Melophagus	2200?	01110	?0101	01100	00111	01101	01101	21011	1111
Crataerina	22010	01110	10111	11100	00110	00101	01111	11011	1111
Stenepteryx	2201?	?1110	?0111	11100	00110	00101	01111	11011	1010
Nycteribosca	22001	11111	00101	11110	02110	10101	01101	01110	1100
Eucampsipoda	22101	1?111	0110-	11111	1-112	11211	11101	2-010	1000

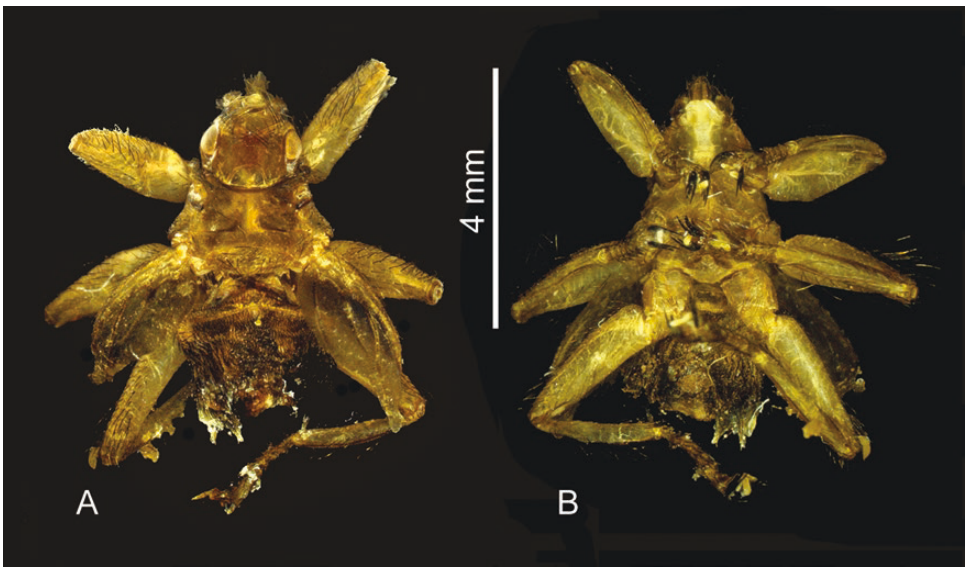

Figure 1. *Crataerina pallida*, digital photographs, habitus. A, dorsal view. B, ventral view.

mer2/3, meso/metacoxal meron; NP, notopleural setae; np, notopleural suture; nt1/3, pro/metanotum; O, origin; PA, post-alar setae; ph1, prophragma; plr2, mesopleural ridge; pls1/2/3, pro/meso/metathoracic pleural suture; pnc, postnotal calli; PP, postpronotal setae; pu, pulvillus; pwp2, mesothoracic pleural wing process; S, scutellar setae; SA, supra-alar setae; scl2, mesoscutellum; scs, subcoxal sclerite; sl, sensillar area; tr1/2/3, pro/meso/metatrochanter; ts, transverse suture; sa2, mesosubalare; sp1/3, pro/metathoracic spiracle; tg, tegula.

RESULTS

THORACIC SKELETON

General features

The thoracic segmental borders appear indistinct in dorsal view (Fig. 1A). The entire thorax is flattened, approximately one-third high as wide. The mesothoracic region is distinctly widened. The dorsal side is largely sclerotized. The head and thorax are tightly connected, together forming a streamlined, compact functional unit; the posterior part of the head covers the dorsal prothorax completely (Fig. 2A, C). The sclerotized dorsal parts of the thorax and the tarsi are brown, whereas the other regions and the proximal parts of the legs display a pale yellowish coloration.

Cervix and prothorax

The cervix and prothorax are dorsally covered by the occipital region of the head. The small triangular first lateral cervical sclerite (lcv1: Fig. 3A) connects the dorsal part of the second lateral cervical sclerite with its tapering posterior part. The second lateral cervical sclerite (lcv2: Figs 3A, 4D) provides a large surface area for the attachment of dorsoventral muscles; in anterior view, it appears curved and narrow; it is not supported by the proepisternum. A pair of tiny sclerotized sensillar areas (sl: Fig. 3A) are located in the membranous area between the ventral parts of the second lateral cervical sclerites.

The very narrow pronotum (nt1: Fig. 3A, B) is concave anteromedially and expanded laterally; it articulates with the lateral margin of the second lateral cervical sclerites with its sharp ventromesal edge. The unpaired prophragma (ph1: Fig. 4D) is suspended below the strongly sclerotized posterior pronotal margin. Very distinct and prominent humeral calli (hc: Fig. 3B) are present at the mesonotal anterolateral corners; they extend anterad and fit tightly with the posterolateral head capsule. A conspicuous, elongated and dorsally oriented spiracle is present posteriorly (sp1: Figs 2A, C, 3B, F). The small propleuron is inserted between the humeral callus dorsally and the procoxa (cx1: Figs 2B, C, 3A, D, F, 4D) on the ventral side. The slightly curved propleural suture (pls1: Fig. 3F) divides the sclerite into the anterior proepisternum (es1: Fig. 3F) and the posterior proepimeron (em1: Fig. 3F). Both articulate with the posterodorsal procoxal margin with their ventral parts. A large membranous area is present between the inner margins of the large procoxae; dorsally, it is connected with the second lateral cervical sclerite; posteriorly, it is connected with the well-developed paired probasisterna (bs1: Fig. 3A, D), which are placed between the posteromesal procoxal edges. Medially, a broad probasisternal apodeme (ma: Fig. 4C) extends posterodorsad. Close to its basal part, a slender and curved profurca

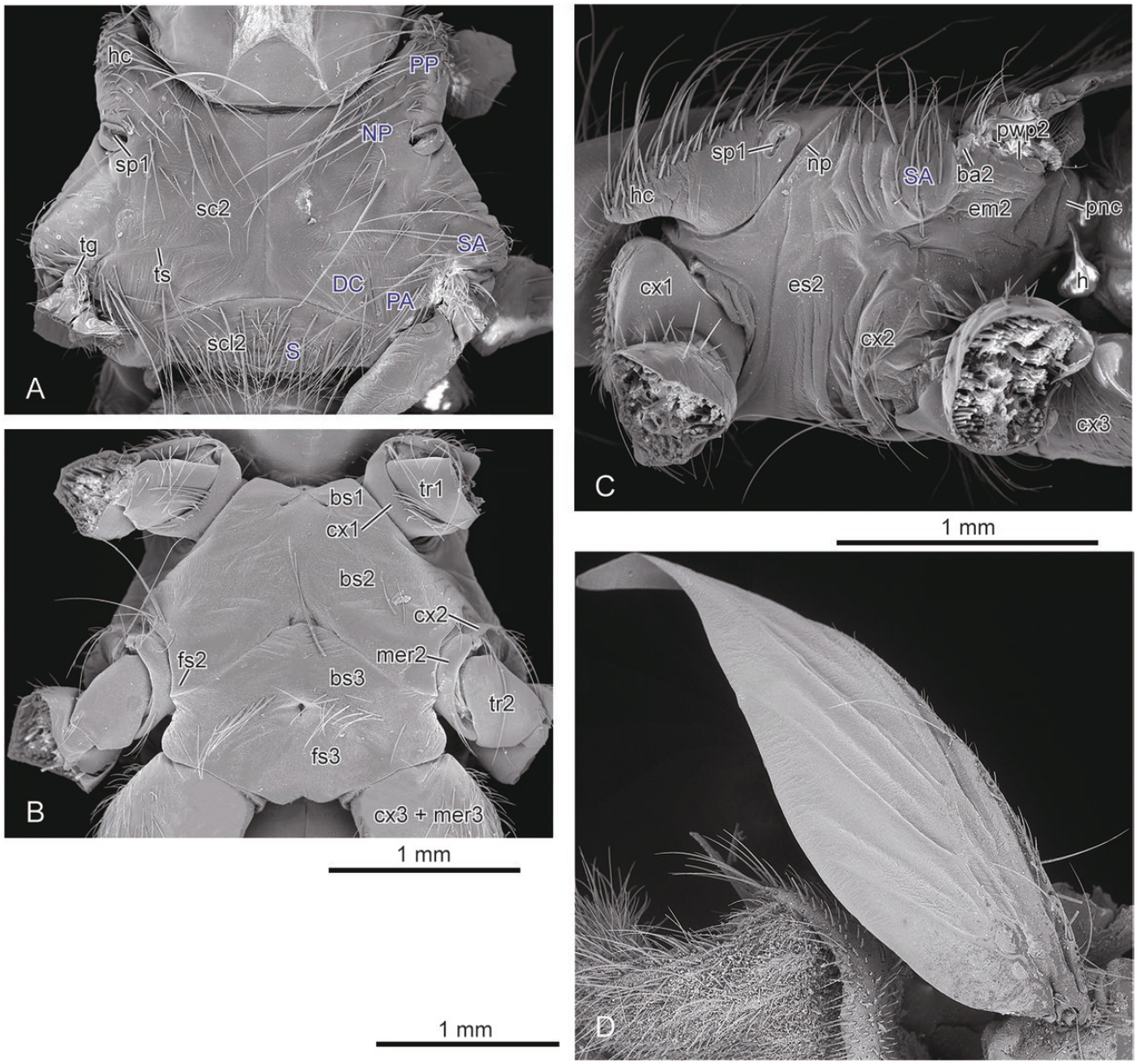

Figure 2. *Crataerina pallida*, scanning electron micrographs, thorax. A, dorsal view. B, ventral view. C, lateral view. D, wing.

(fu1: Fig. 4D) extends posterodorsad; it is connected with the propleural suture.

Mesothorax

The very broad mesoscutum (sc2: Figs 2A, 3A, B) is longitudinally subdivided by a distinct median suture. A pair of transverse sutures (ts: Fig. 3B) on the posterior mesoscutal third partly divides the sclerite in the transverse direction. The notopleural suture is distinct and slightly curved (np: Figs 2C, 3B); it extends from the lateral edge of the middle region of the mesoscutum to the mesopleuron. The flat triangular mesoscutellum (scl2: Figs 2A, 3B, C) is attached to the posterior mesoscutal margin; its lateral arm is extended to form the posterior margin of the wing base. The postnotum (pn2: Fig. 3C) appears as a curved transverse band below the posterior scutellar edge. Laterally, a pair of large bulges forms the postnotal calli (pnc: Figs 2C, 3C, F).

The mesepisternum is very large (es2: Figs 2C, 3F); a broad anterior process connects it with the posterior proepimeral margin; dorsally, a long and curved suture separates it from the humeral callus and mesoscutum. The mesobasalare (ba2: Figs 2C,

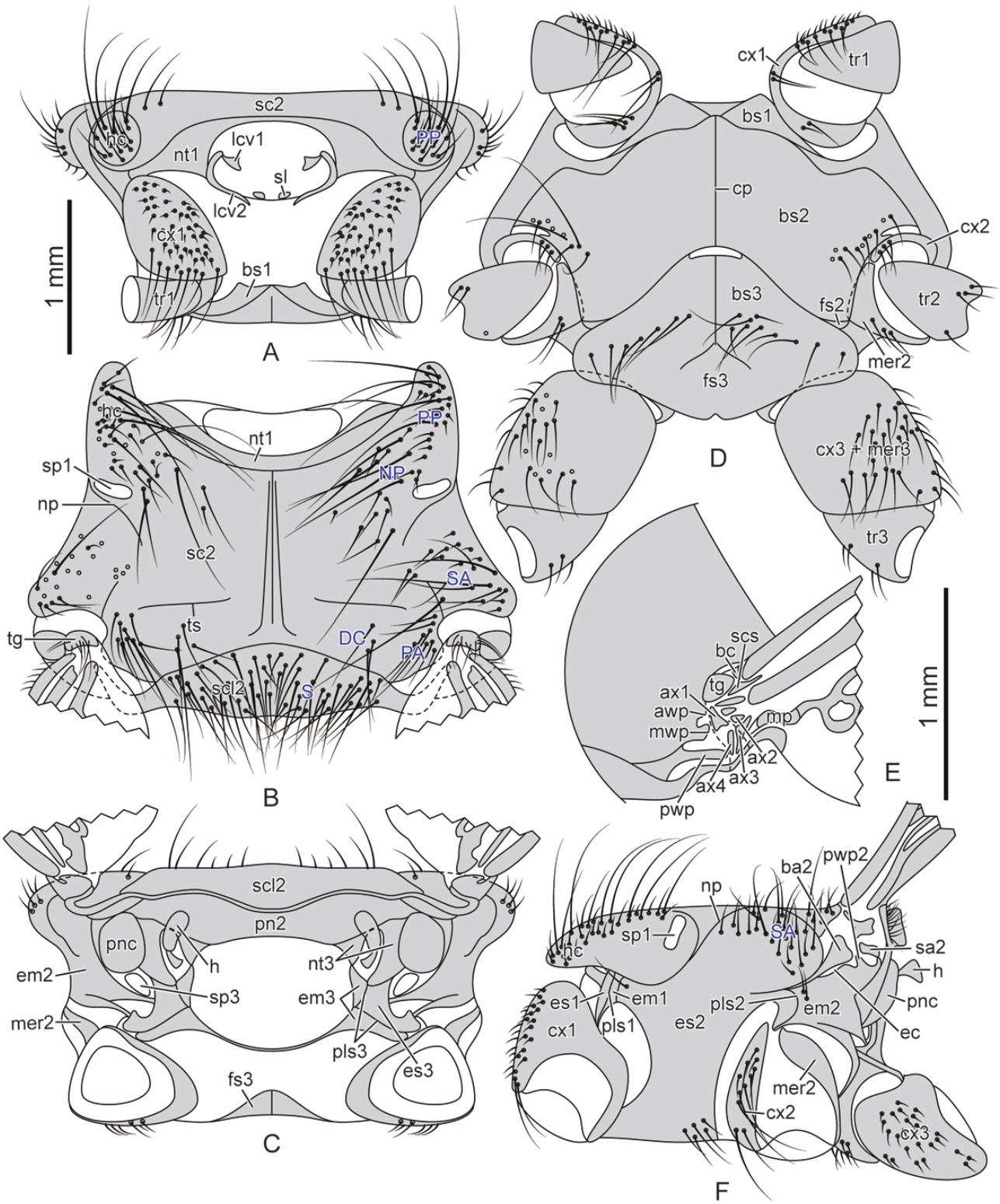

Figure 3. *Crataerina pallida*, line drawing, thoracic skeleton. A, frontal view. B, dorsal view. C, posterior view. D, ventral view. E, wing base. F, lateral view. Scale bars: upper bar 1 mm in A–D, F; lower bar 1 mm in E.

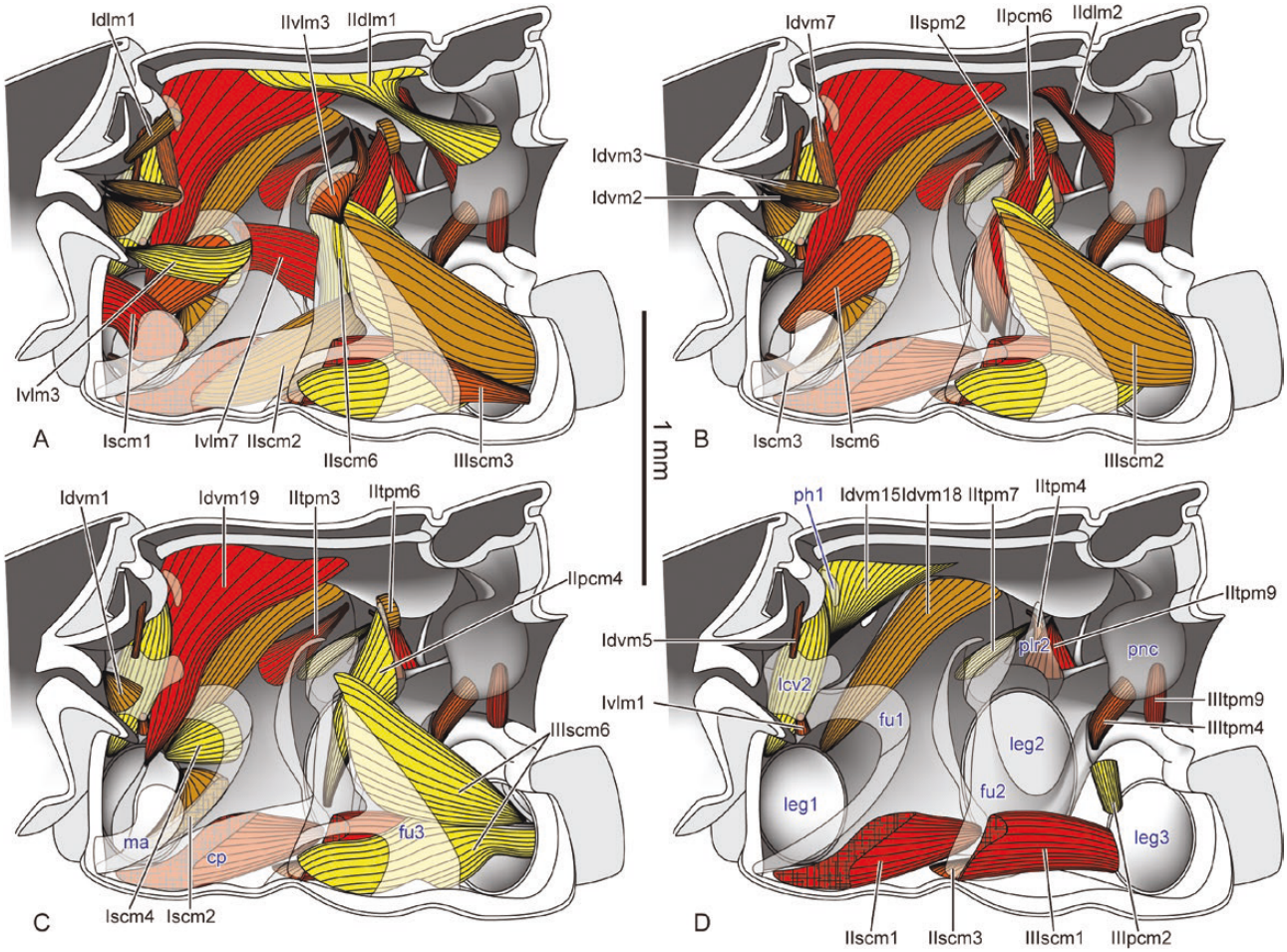

Figure 4. *Crataerina pallida*, line drawing (based on 3D-reconstructions), thoracic endoskeleton and muscles. Lateral view. Skeletal structures are labelled in blue, muscles in black. The muscles are removed layer by layer from A to D.

3F) extends from the posteromedian mesepisternal margin; it is enlarged dorsally; a distinctly developed basalar apodeme is lacking. The curved mesothoracic pleural suture (pls2: Figs 2C, 3F) separates the mesepimeron (em2: Figs 2C, 3C, F) from the anterior mesepisternum (es2: Figs 2C, 3F). The former connects posterodorsally with the lateral postnotal margin. The mesepisternal cleft (ec: Fig. 3F) also separates the mesepimeron from the anterior mesobasalare. Dorsally, the mesothoracic pleural wing process (pwp2: Figs 2C, 3F) is present as a tiny process, close to the dorsal mesosubalare (sa2: Fig. 3F). The triangular mesopleural ridge (plr2: Fig. 4D) extends along the mesopleural suture above the mesocoxal rim. The mesopleural suture articulates ventrally with the elongated mesocoxa (cx2: Figs 2B, C, 3D, F) and mesomeron (mer2: Figs 2B, C, 3D, F). Together, these proximal leg elements form a wide articulatory area for the mesotrochanter (tr2: Fig. 3D).

The mesobasisternum (bs2: Figs 2B, 3D) is large and broadly fused with the mesepisterna laterally. The elongated triangular cryptosternum (cp: Fig. 4C) extends into the thoracic lumen from the external median suture; its posterior part is divided into two branches, each of them connected to the mesofurca on the respective side. The mesofurcasternum (fs: Fig. 3D) is a small area behind the mesobasisternal posterior corner. The strongly developed mesofurca (fu2: Fig. 4D) extends along the mesobasisternal posterior margin; dorsally, it forms an apical disc; laterally, a thick, curved arm connects it to the mesepimeron.

Metathorax

The metanotum (nt3: Fig. 3C) is very small compared with the mesonotum and widely separated into two parts by the median intersegment between the thorax and abdomen. Both metanotal halves are dorsally fused with the ventrolateral postnotal margin. Each of

them is almost completely divided by a deep, vertically oriented concavity, where the halteres are inserted. The halteres (h: Figs 2C, 3C, F) are distinctly developed, with a conical base, a short stalk and an oval knob-like distal part. The metepisternum is dorsally connected with the ventral metanotal margin and narrows ventrally towards the metacoxal rim. Anteriorly, the metathoracic spiracle (sp3: Fig. 3C) is embedded in the membranous area below the postnotal callus. It is shorter than its prothoracic counterpart. The curved metathoracic pleural suture (pls3: Fig. 3C) divides the metepimeron (em3: Fig. 3C) into two parts. The laterally extended ventral part reaches the metacoxal rim, and a thin mesal projection is medially fused to the corresponding metepimeral element of the other side. The very large metacoxae include a well-developed metameron (cx3 + mer3: Figs 2B, 3D). The anterior margin of the wide metabasisternum (bs3: Figs 2B, 3D) projects into the posterior concavity of the mesobasisternum. Its posterior area is completely fused with the metafurcasternum (fs3: Fig. 3C, D). The large triangular metafurca (fu3: Fig. 4C) extends along the median line; its anteromedian process is attached to the posteromedian margin of the cryptosternum.

Legs

The legs are strongly developed. The distal elements of all three pairs are similar (see hindleg: Fig. 5A–C), but the coxae differ distinctly in shape, size and articulation. The procoxae (cx1: Figs 2B, C, 3A, D, F, 4D) appear inflated and are freely movable in all directions; a distinct, slightly rounded vertical edge is present anterolaterally; a dense field of short spines is inserted on the anterior margin and anterior surface. The roughly triangular protrochanter (tr1: Figs 2B, 3A, D) is inserted on a wide membranous area of the distal procoxal part; its distal part is movably connected with the profemoral base. The profemur is almost straight, with nearly parallel anterior and posterior margins and an oblique distal edge; it bears a distinct vestiture of longer setae, mainly concentrated on the distal part, and shorter setae on the anterior area of the proximal region. The protibia is about as long as the profemur, narrower, slightly curved basally and widening distally; the vestiture of setae on the protibia is sparse compared with that of the profemur; a row of long setae is present on the middle region of the dorsal surface; shorter setae are inserted along the anterior margin. The protarsomeres 1–4 are short and moderately flattened; protarsomere 4 is apically emarginated; protarsomere 5 is large, about as long as 1–4 combined, also flattened, and distinctly widened distally. All protarsomeres bear an area of short setae; two extensive setose fields cover almost the entire surface of protarsomeres 1–4, leaving only a narrow stripe on the dorsal and ventral side glabrous; protarsomere 5 bears only three pairs of setae on the dorsal surface, one pair at the edges and two closely adjacent pairs on the middle region of the distal edge; very few setae are inserted on the lateral surface, and two fields of setae are present on the ventral side: one on the ventrolateral edges and one apically close to the empodium. The empodium is about half as long as protarsomere 5, slender and slightly narrowing distally; rows of very short setae are inserted along the edges and on the ventral side of the distal two-thirds; all setae are directed towards the apex; the base of the empodium is glabrous. The pulvilli are flexible structures, covered with minute hairs (Richards & Richards, 1979: acanthae) on the ventral surface. The claws are strongly developed, both forming curved bifid structures, appearing tridentate together with the strongly developed curved and heel-like claw tooth; very fine grooves are discernable on the internal surface of the claws.

The mesocoxae (cx2: Figs 2B, C, 3D, F) are smaller than their prothoracic counterparts and restricted in their movability; in contrast to Schlein (1970), they contain a narrow sclerotized mesomeron (mer2: Figs 2B, C, 3D, F). The metacoxae (cx3: Figs 2B, C, 3D) contain a large metameron (mer3: Figs 2B, 3D); they are distinctly larger than the procoxae and largely or completely immobilized at their base. The meso- and metafemora (metafemur: Fig. 5A–C) are slightly curved posterad, and slightly more elongated than their prothoracic counterparts. The tibiofemoral joint of the midleg is more strongly pronounced than that of the foreleg, and even more conspicuous on the hindleg. The metatibiae are slightly more elongated than the pro- and mesotibiae.

Mesothoracic wing (Fig. 2D) and wing base (Fig. 3E)

The lancet-shaped forewings are distinctly shortened, measuring 59% of the total body length from the base to the apex in males and 77% in females (Massonat, 1909: fig. 16). The venation is distinctly modified, concentrated in the anterior region of the proximal half. Four longitudinal veins reach the hind margin. Only two cross veins are present.

The wing base sclerites are largely merged with the membranous dorsolateral region of the segment. Consequently, their shape and configuration are difficult to trace. The slightly bulging mesothoracic tegula (tg) is distally surrounded by the basicosta (bc) and the narrow subcoxal sclerite (scs). The triangular first axillary sclerite (ax1) articulates proximally with the blunt anterior notal wing process (awp) and with the median notal wing process (mwp). Distally, the second axillary sclerite (ax2) articulates with the anterior margin of the elongated third axillary sclerite (ax3).

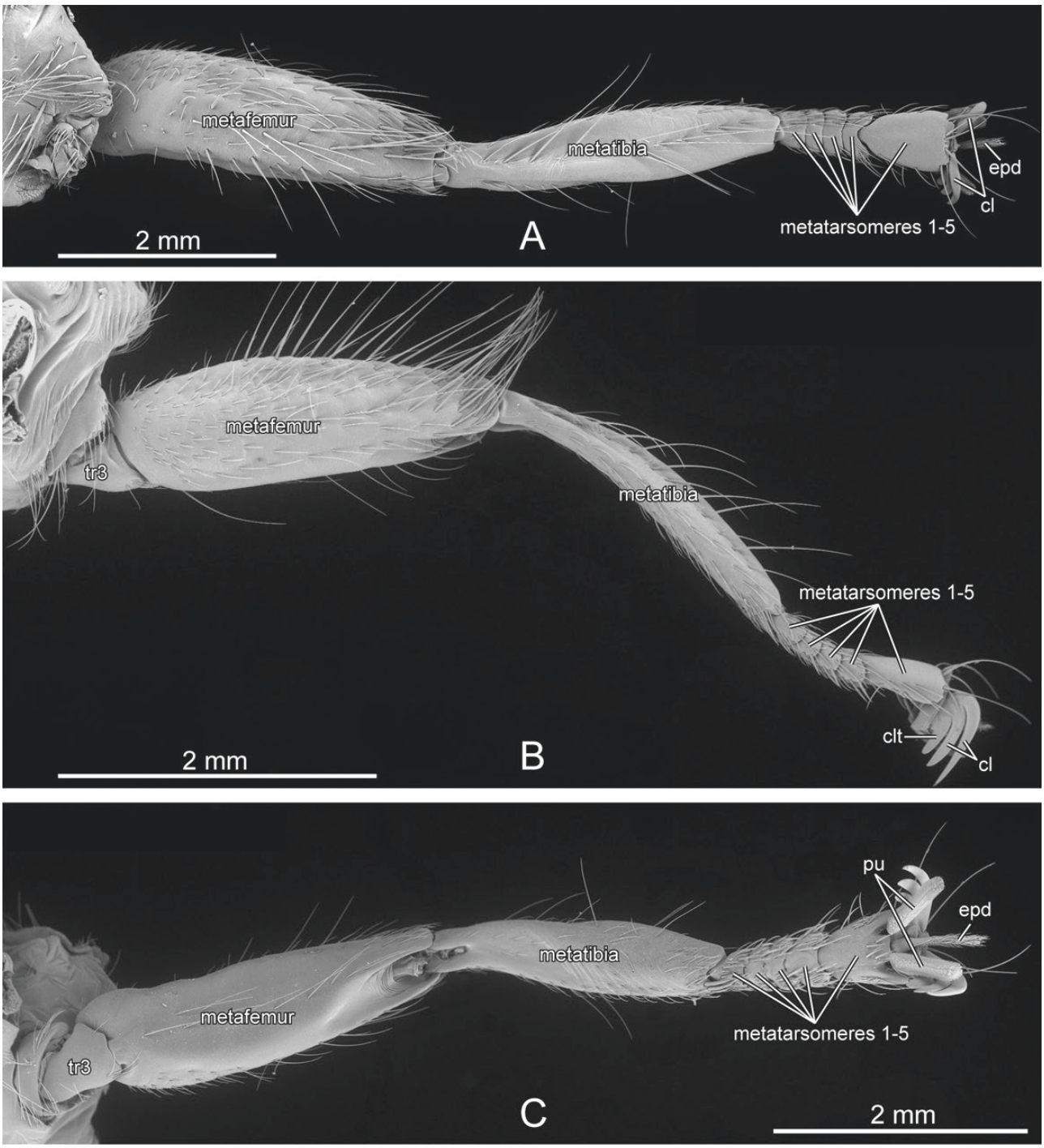

Figure 5. *Crataerina pallida*, scanning electron micrographs, hindlegs. A, dorsal view. B, lateral view. C, ventral view.

The proximal margin of the latter is close to the narrow fourth axillary sclerite (ax4). The posterior notal wing process (pwp) is slender proximally.

Metathoracic haltere

The short stalks of the spoon-shaped halteres (h: Figs 2C, 3C, F) are located in a membranous area surrounded by the posterolateral parts of the metanotum.

Chaetotaxy

The postpronotal (PP: Figs 2A, 5A, B) and notopleural setae (NP: Figs 2A, 3B) form a continuous cluster from the humeral callus to the mesonotal anterolateral

area. The wing base is surrounded by the supra-alar (SA: Figs 2A, C, 3B) and post-alar setae (PA: Figs 2A, 3B); the former also extend to the dorsal mesepisternal area. Only three or four post-sutural dorsal setae (DC: Figs 2A, 3B) are present in the central area between the transverse suture and the mesonotal posterior margin. The scutellar setae (S: Figs 2A, 3B) densely cover the entire mesoscutellum.

Thoracic musculature

Prothorax

Dorsal longitudinal muscle: **Idlm1**M. prophragma-occipitalis; O (= origin): prophragma; I (= insertion): dorsal area of occipitale.

Dorsoventral muscles: **Idvm1** M. cervico-occipitalis anterior, conical, broader at attachment area on cervical sclerite, narrowing towards insertion on head capsule; O, anteroventral area of second lateral cervical sclerite; I, posteromedian area of occipital region of head capsule. **Idvm2** M. cervico-occipitalis medialis, slightly bent upwards, narrowing towards insertion; O, posterodorsal area of second lateral cervical sclerite; I, posteromedian area of occipital region. **Idvm3** M. cervico-occipitalis posterior, narrowing towards occipital; O, posterodorsal area of second lateral cervical sclerite; I, posteromedian area of occipital region. **Idvm5** M. pronoto-cervicalis anterior; O, median area of pronotum; I, anterodorsal area of second lateral cervical sclerite. **Idvm7** M. pronoto-cervicalis posterior; O, posteromedian pronotal margin; I, posterodorsal area of second lateral cervical sclerite. **Idvm15** M. pronoto-trochantinocoxalis, very large, broader on area of origin, narrowing towards insertion; O, anteromedian area of mesoscutum; I, anterodorsal procoxal rim. **Idvm18** M. pronoto-coxalis lateralis, very large, broader on area of origin, narrowing towards insertion; O, mesolateral area of mesoscutum; I, posterodorsal procoxal rim. **Idvm19** M. pronoto-trochanteralis, very large, broader on area of origin, narrowing towards insertion; O, anterior half area of mesoscutum; I, protrochanteral tendon.

Ventral longitudinal muscles: **Ivlm1** M. profurca-cervicalis, very short; O, ventrodistal area of profurca; I, posteroventral area of second lateral cervical sclerite. **Ivlm3** M. profurca-tentorialis, triangular, broader on profurcal area of origin, narrowing towards insertion on head capsule; O, dorsal side of dorsal area of profurca; I, posterior occipital area. **Ivlm7** M. profurca-mesofurcalis, broad; O, ventral side of dorsal area of profurca; I, anteromedian area of mesofurca.

Sterno-coxal muscles: **Iscm1** M. profurca-coxalis anterior, large; O, dorsal area of median apodeme of probasisternum; I, anterior procoxal rim. **Iscm2** M. profurca-coxalis posterior, broadly triangular, broader on profurcal area of origin, narrowing towards insertion; O, ventroproximal area of profurca; I, posterodorsal procoxal rim. **Iscm3** M. profurca-coxalis medialis, slightly bent; O, proximal region of profurca, close to the base; I, anteroventral procoxal rim. **Iscm4** M. profurca-coxalis lateralis, wide, triangular, broader on profurcal area of origin, narrowing towards insertion; O, ventral side of distal part of profurca; I, posterodorsal procoxal rim. **Iscm6** M. profurca-trochanteralis, broader on area of origin, narrowing towards insertion; O, dorsal side of upper part of profurca; I, protrochanteral tendon.

Mesothorax

Dorsal longitudinal muscles: **IIdlm1** M. prophragma-mesophragmalis, narrow on postnotal callus, very broad on mesoscutum; O, posterior half area of mesoscutum; I, postnotal callus. **IIdlm2** M. mesonoto-phragmalis; O, posterolateral area of mesoscutum; I, postnotal callus.

Tergo-pleural muscles: **IItpm1** M. prophragma-mesanepisternalis, broader on mesepisternum, narrowing towards mesoscutum; O, posterolateral area of mesoscutum; I, posteromedian area of mesepisternum m. **IItpm4** M. mesonoto-pleuralis anterior, small; O, posteromedian area of mesopleural ridge; I, first axillary sclerite. **IItpm6** M. mesonoto-pleuralis posterior, short and thick; O, posterodorsal area of mesopleural ridge; I, posterolateral mesoscutal margin. **IItpm7** M. mesanepisterno-axillaris; O, posteromedian area of mesepisternum; I, third axillary sclerite. **IItpm9** M. mesepimero-axillaries tertius, conical, broader on mesepimeron, narrowing towards third axillary sclerite; O, mesepimeron close to base of mesopleural ridge; I, third axillary sclerite.

Sterno-pleural muscles: **IIspm2** M. mesofurca-pleuralis, slightly bent; O, distally on dorsal side of mesofurca; I, dorsal part of mesothoracic pleural suture.

Pleuro-coxal muscles: **IIpcm4** M. mesanepisterno-coxalis posterior, very large, broader on area of origin, narrowing towards insertion; O, anterior area of mesopleural ridge; I, anteroventral mesocoxal rim. **IIpcm6** M. mesopleura-trochanteralis, very large, narrowing towards insertion; O, anterodorsal area of mesopleural ridge; I, mesotrochanteral tendon.

Ventral longitudinal muscle: **IIvlm3** M. mesofurca-metafurcalis, conical, broader on metafurca, narrowing towards mesofurca; O, posterodorsal area of mesofurca; I, dorsal tip of metafurca.

Sterno-coxal muscles: **IIscm1** M. mesofurca-coxalis anterior, very large, broader on are of origin, narrowing towards insertion; O, anterior area of cryptosternum; I, anteroventral mesocoxal rim. **IIscm2** M. mesofurca-coxalis posterior, very large, narrowing towards insertion; O, median area of cryptosternum; I, mesocoxal posterior rim. **IIscm3** M. mesofurca-coxalis medialis, broader on are of origin, narrowing towards insertion; O, posteromedian area of mesobasisternum; I, ventral mesocoxal rim. **IIscm6** M. mesofurca-trochanteralis, strongly developed; O, dorsodistal area of mesofurca; I, mesotrochanteral tendon.

Metathorax

Tergo-pleural muscles: **IIItpm4** M. metanoto-pleuralis anterior; O, metepisternum; I, basally on haltere. **IIItpm9** M. metepimero-axillaris tertius; O, dorsal part of metepimeron; I, basally on haltere.

Pleuro-coxal muscle: **IIIpcm2** M. metabasalare-trochantinalis; O, metepisternum; I, anterodorsal metacoxal rim.

Sterno-coxal muscles: **IIIscm1** M. metafurca-coxalis anterior, very strongly developed; O, anteromedian process of metafurca; I, anteroventral metacoxal rim. **IIIscm2** M. metafurca-coxalis posterior, very large; O, anterodorsal area of metafurca; I, posterodorsal metacoxal rim. **IIIscm3** M. metafurca-coxalis medialis, broader on area of origin, narrowing towards insertion; O, posteroventral area of metafurca; I, posteroventral metacoxal rim. **IIIscm6** M. metafurca-trochanteralis, two large bundles; O, anterodorsal metafurcal margin and median area of metabasisternum; I, metatrochanteral tendon.

Owing to the strongly reduced metathorax, the homology of the three pleural muscles (IIItpm4, IIItpm9 and IIIpcm2) is difficult to assess. Our provisional homology assessment is based on their relative positions.

Phylogenetically relevant characters of the thorax

In this section, thoracic characters of *C. pallida* and other representatives of Hippoboscoidea are listed and discussed. Based on the observations presented here and published morphological information (e.g. Massonat, 1909; Bequaert, 1953; Schlein, 1970), the characters are coded for eight selected hippoboscoid taxa and two outgroup terminals with available detailed data. The characters were analysed cladistically as outlined in the Material and methods.

General thoracic characters

1. Thoracic segmental borders on dorsal side: (0) distinctly separated segments; (1) indistinct; (2) segmentation almost completely obliterated. A characteristic feature of Pupipara is the far-reaching obliteration of the dorsal segmental borders of the thorax (Massonat, 1909: 'aucune trace de segmentation', pls. I–VII; Bequaert, 1953; Zeve & Howell, 1963: figs 1–6; Schlein, 1970). The three segments form a compact ensemble, also allowing unusual shifts of muscles. The dorsal thoracic division is less indistinct in *Ornithoica* (Ornithoicinae) than in other hippoboscids (Bequaert, 1953).
2. Dorsoventral compression of thorax: (0) not flattened; (1) moderately flattened; (2) strongly flattened, height less than half the width of the mesonotum. Usually distinctly flattened in Pupipara, often only one-third as high as mesonotal width (Massonat, 1909; Bequaert, 1953: fig. 8A; Schlein, 1970; Maa & Peterson, 1987; Wenzel & Peterson, 1987). Less compressed dorsoventrally in *Ornithoica* (Bequaert, 1953: 'less depressed than that of the other subfamilies…'; Hennig, 1973). Varying in Streblidae from subglobose to strongly flattened (Streblinae) or laterally compressed and flea-like (Nycteribiinae) (Wenzel & Peterson, 1987). About as high as mesonotal width in Glossinidae (Bequaert, 1953: fig. 8B) and other groups of Diptera (Hennig, 1973). Schlein (1970) suggested a correlation between flattened thoracic segments and bent mesothoracic pleural sutures with two sharp edges. However, this interpretation is questionable. A similar feature also occurs in other dipteran families with a largely unmodified thorax (Bonhag, 1949: Tabanidae; Schlein, 1970: Muscidae).
3. Sclerotization of dorsal thoracic surface: (0) largely or completely sclerotized; (1) rudimentary. A largely unsclerotized thoracic dorsum (Nußbaum, 1960: fig. 2; Schlein, 1970) is likely to be an autapomorphy of Nycteribiidae.
4. Connection of head and thorax: (0) both not forming a tight unit; (1) head fitting tightly into thorax, both forming a streamlined unit. The head of *Crataerina* and *Stenepteryx* fits tightly with the anterior thorax, both forming a compact, streamlined unit (Bequaert, 1953). A close cephalo-thoracic connection is characteristic for ectoparasites of birds and is most perfect in species using fast-flying swallows or swifts as hosts.

Cervix and prothorax

5. Sensillar area of cervical region: (0) one plate; (1) two plates. Two plates are present in the membranous area between the ventral parts of the second lateral cervical sclerites of *Crataerina*. Two are also present in *Hippobosca* and *Musca*, but only one in Glossinidae, Streblidae and Nycteribiidae (Schlein, 1970).

6. First lateral cervical sclerites: (0) present; (1) absent. Normally present, but missing in *Nycteribosca* and other Streblidae, and also in Nycteribiidae (Bequaert, 1953; Schlein, 1970; Hennig, 1973;).
7. Proepisternal support of second lateral cervical sclerite: (0) present; (1) reduced. The pleural support of the second cervical sclerite is reduced in *Nycteribosca* and other Streblidae (Schlein, 1970; Hennig, 1973). It is present in *Hippobosca* but absent in other Hippoboscidae, including *Crataerina*. Also present in *Musca* (Schlein, 1970).
8. Protergum: (0) normally developed; (1) strongly shortened. Generally, strongly shortened in Pupipara (Bequaert, 1953).
9. Space between procoxae: (0) procoxae adjacent or nearly adjacent medially; (1) separated by approximately one-third of coxal width, without large membranous area; (2) widely separated, with large membranous area between them. Usually more or less contiguous in Diptera (Hennig, 1973). Moderately widely separated in Glossina (Bequaert, 1953: fig. 9B). Widely separated in Pupipara (Bequaert, 1953: fig. 9A). Narrowly separated in Glossinidae.
10. Probasisternum: (0) distinctly developed; (1) largely reduced or absent as a recognizable separate structure. Vestigial in Streblidae and largely or completely reduced in Nycteribiidae (Schlein, 1970).
11. Median apodeme of probasisternum: (0) absent; (1) present. A median apodeme is present in *Glossina*, *Hippobosca* and *Crataerina*. It is missing in *Drosophila* and *Musca*, and also in Streblidae and Nycteribiidae (Schlein, 1970).
12. Tube-like sensory organ of prosternal region: (0) absent; (1) present. Present in Nycteribiidae (Schlein, 1970).

Mesothorax

13. Shape of mesothorax: (0) as long as or longer than wide; (1) wider than long. Almost generally wider than long in Pupipara (Massonat, 1909: pls. I–VI; Bequaert, 1953: fig. 8A), but laterally compressed in some Streblidae (Wenzel & Peterson, 1987: Nycterophiliinae). Longer than wide in Glossinidae (Bequaert, 1953: fig. 8B).
14. Shape of humeral callus: (0) not distinctly protruding; (1) distinctly protruding and pointed. Very prominent in *Crataerina* and other Ornithomyiinae (Hennig, 1973), but indistinct in *Ornithoica* (Hennig, 1973) and Lipopteninae, and slightly projecting in *Hippobosca* (Bequaert, 1953). The posthumeral border of the humeral callus is obliterated in Streblidae (Schlein, 1970). Even though the humeral calli and the adjacent spiracles are completely fused to the mesonotum, they are likely to be derived from the posterior pronotal area. This is suggested by a comparison with the pronotal subdivision in Tipulidae (Matsuda, 1970) and by postpronotal setae covering the humeral calli.
15. Median suture dividing mesonotum: (0) absent; (1) present. Present and dividing mesonotum in *Crataerina* and most other Hippoboscidae, and also distinct in Streblidae (Massonat, 1909: figs 24, 30, 33, 36, 40, 46; Bequaert, 1953: fig. 8A; Zeve & Howell, 1962: figs 1, 2). Short in some species of *Lipoptena* (Bequaert, 1953: fig. 11A) and indistinct but still recognizable in *Stenepteryx* (Massonat, 1909: fig. 54). Absent in Nycteribiinae (Peterson & Wenzel, 1987) and Glossinidae. Absent in *Melophagus* (Massonat, 1909: fig. 20) and *Ornithoica* (Maa, 1966: figs 4, 5). Inapplicable (coded as [-]) in Nycteribiidae due to unsclerotized thoracic dorsum (Nußbaum, 1960).
16. Mesepisternal ridge: (0) developed; (1) reduced. The mesepisternal ridge is reduced in *Glossina* and Pupipara (Schlein, 1970).
17. Space between mesocoxae: (0) narrowly separated; (1) widely separated. Very widely separated in Pupipara (Hennig, 1941; Bequaert, 1953: fig. 9a; Schlein, 1970: figs 23, 28A). Narrowly separated in Glossinidae.
18. Size of mesobasisternum: (0) moderately sized; (1) greatly expanded. Very extensive in *Crataerina* and other groups of Pupipara (Bequaert, 1953; Schlein, 1970).
19. Anterior end of mesobasisternum: (0) not folded upwards and backwards; (1) folded upwards and backwards. Folded upwards and backwards in Streblidae and Nycteribiidae, displacing the profurca on a second plane (Schlein, 1970).
20. Mesofurca: (0) well developed; (1) reduced. The mesofurca is reduced to a small stalk in Nycteribiidae (Schlein, 1970).
21. Cup-shaped upper end of mesofurca: (0) present; (1) absent. The cup-shaped upper end of the mesofurca, which normally serves for attachment of flight muscles, is missing in Nycteribiidae (Schlein, 1970).

Metathorax

22. Transverse suture of metanotum: (0) present; (1) small triangular sclerites above halteres; (2) with two lateral posterior lobes. Metanotum represented by transverse suture in Hippoboscidae (Schlein, 1970) and *Drosophila* (Fabian *et al.*, 2016), but by small triangles above the halteres in *Musca* and *Glossina*. With two lateral posterior lobes in *Nycteribosca* (Streblidae) (Schlein, 1970).

23. Ventral connection of metepimera: (0) separated; (1) fused ventrally. Fused ventrally in Hippoboscidae, Streblidae and Nycteribiidae (Schlein, 1970).
24. Space between metacoxae: (0) narrowly separated; (1) widely separated. Usually contiguous in Diptera (Schlein, 1970; Fabian *et al.*, 2016) but widely separated in Pupipara (Massonat, 1909; Hennig, 1941; Bequaert, 1953: fig. 9A; Nußbaum, 1960: fig. 2; Schlein, 1970). Narrowly separated in Glossinidae.
25. Shape of metafurcasternum: (0) straight or only moderately curved; (1) s-shape; (2) z-shape. S-shape in *Melophagus* and z-shape in *Nycteribiidae*, correlated with increased flattening of the thorax (Schlein, 1970).
26. Paired metanotal process: (0) absent; (1) present. Present and projecting into the abdomen in Streblidae and Nycteribiidae (Schlein, 1970). Possibly related with pupiparity according to Schlein (1970).

Legs

27. Position of coxae: (0) ventral; (1) dorsal. All coxae of *Melophagus* and Nycteribiidae are shifted onto the dorsal side of the body (Massonat, 1909: fig. 14; Nußbaum, 1960; Wenzel & Peterson, 1987: fig. 112.3).
28. Shape of procoxa: (0) not inflated; (1) inflated; (2) elongated and nearly cylindrical. Inflated and freely movable in different directions in *Crataerina* and members of Hippoboscidae (Bequaert, 1953: fig. 8a). According to Massonat (1909), the robustness ('robustesse') of the coxa increases with the degree of fixation to the host. More elongated and nearly cylindrical in *Basilia* (Nycteribiidae) (Nußbaum, 1960: fig. 2).
29. Mesocoxal ctenidium: (0) absent; (1) present. A thoracic ctenidium with a row of strongly developed, curved spines is formed as an elongation of the mesocoxa of Nycteribiidae (Schlein, 1970). This feature is apparently an autapomorphy of this family. It can be moved by a leg muscle (Schlein, 1970: fig. 23; Hennig, 1973) and is used to improve the anchorage in the fur of the bat.
30. Movability of mesotrochanter: (0) mainly between trochanter and coxa; (1) movably connected with coxa and femur. An increased movability between mesotrochanter and mesofemur in Pupipara is arguably linked with an increased fixation of the mesocoxa (Bequaert, 1953).
31. Shape of protarsomere 1: (0) not elongated and curved; (1) elongated and curved. Very distinctly elongated and curved in *Eucampsipoda hyrtli* and other nycteribiids examined.
32. Shape of tarsomeres 2–4: (0) longer than wide; (1) as long as wide or shorter. Tarsomeres 2–4 are distinctly shortened and compact in Pupipara (Massonat, 1909: fig. 22, pls. I, III, IV, VI, figs 10, 24, 32, 48; Bequaert, 1953: fig. 12C, D; Maa & Peterson, 1987; Wenzel & Peterson, 1987).
33. Broad flattened heel of claws: (0) absent or heel weakly developed; (1) distinctly developed. Claws with a conspicuous, broadened and flattened base or heel ('Krallengelenkhöcker') are characteristic for Pupipara (Massonat, 1909: fig. 21; Bequaert, 1953: figs 12F, K).
34. Division of claws: (0) undivided claws; (1) claws bifid (longitudinally split). Two longitudinal separate subunits of each claw are characteristic for ectoparasites of birds (Massonat, 1909: *Crataerina*, *Stenepteryx*) with the noteworthy exception of *Ornithoica*. Together with the curved claw-like claw tooth, they form a tridentate structure (Bequaert, 1953: fig. 12A).
35. Pulvillus: (0) oval or widening distally and densely set with adhesive hairs (acantae without sockets); (1) cylindrical or distally narrowing, elongated and soft, with distinctly reduced vestiture of short hairs. Oval or distally widening and densely set with adhesive hairs in many groups of Diptera, including *Glossina* (Bauchhenß, 1979; Gorb, 1998; Beutel & Gorb, 2001; Friedemann *et al.*, 2014: fig. 8). Elongated and modified as soft structures in Pupipara (Bequaert, 1953; Nußbaum, 1960: fig. 18). Usually cylindrical, but distally narrowing in some Streblidae (Zeve & Howell, 1963: fig. 38). Short adhesive hairs are present, but the vestiture appears sparse compared with other groups of Diptera (Bequaert, 1953; Nußbaum, 1960; Friedemann *et al.*, 2014).

Wings and associated structures

36. Mesothoracic wings: (0) normally developed, permanent or shed after short flight; (1) partly reduced, shortened and lancet shaped; (2) completely absent. Shortened, lancet shaped and not suitable for active flight in *Crataerina* and *Stenepteryx* (Massonat, 1909). Also showing different degrees of reduction in various other representatives of Hippoboscoidea; for instance, in most Streblidae (Wenzel & Peterson, 1987). Always absent in Nycteribiidae and also missing in *Melophagus*, but present in 'volant individuals' of related genera (Bequaert, 1953). Also completely reduced in the non-related bee parasite *Braula* (Braulidae) (Massonat, 1909; Bequaert, 1953; Hennig, 1973). Normally developed in Glossinidae and the majority of the other hippoboscoid taxa, including some species of Streblidae (Schlein, 1970: *Nycteribosca aluaudi*; Wenzel & Peterson, 1987).

37. Elongated arm of posterior notal wing process: (0) present and connected with mesonotum; (1) absent. Absent in Hippoboscidae and Streblidae (Schlein, 1970).
38. Club-shaped projection of the third axillary sclerite (calypteron): (0) absent; (1) present. A conspicuous club-shaped projection of the third axillary sclerite is present in Streblidae, but absent in all other groups under consideration (Schlein, 1970).
39. Mesobasalar apodeme: (0) present; (1) partly or completely reduced. Present in *Drosophila* (Fabian *et al.*, 2016: basalar apophysis) and *Musca* (Schlein, 1970). Distinctly reduced or absent in *Glossina* and Hippoboscidae, and also missing or vestigial in Streblidae and Nycteribiidae (Schlein, 1970).

Muscles

40. Origin of prothoracic dorsoventral muscles: (0) at least some on pronotum; (1) all on mesonotum. The shift of all prothoracic dorsoventral muscles onto the mesonotum (or mediotergal plate) is a characteristic feature of Hippoboscidae (Fig. 5C, D) and Nycteribiidae (Nußbaum, 1960: fig. 20, 21). The shifted origins are probably attributable to the limited space on the pronotum. Sufficient attachment areas for the leg muscles are available on the mesonotum. The control of the forelegs is important for the fixation and locomotion on the host.
41. M. pronoto–coxalis lateralis (Idvm18): (0) present; (1) absent. Present and strongly developed in *Crataerina*. Also present in other groups of Pupipara and in Muscidae (Schlein, 1970). Missing in *Glossina* and *Drosophila* (Schlein, 1970; Fabian *et al.*, 2016).
42. M. procoxa-cervicalis (Ipcm1): (0) present; (1) absent. Absent in *Crataerina*, *Melophagus* and Streblidae. Present in other members of Hippoboscidae and in Nycteribiidae (Schlein, 1970).
43. M. mesonoto-trochanteralis (IIdvm7): (0) present; (1) absent. The mesotergal depressor of the mesotrochanter is absent in Glossinidae and Hippoboscidae, but present in Streblidae and Nycteribiidae (Schlein, 1970; Hennig, 1973).
44. M. metanoto-sternalis (IIIdvm1): (0) present; (1) absent. Absent in *Drosophila*, *Crataerina* and *Melophagus* (Schlein, 1970; Fabian *et al.*, 2016). Present in other Hippoboscidae, including *Stenepteryx*, and also in Streblidae and Nycteribiidae (Schlein, 1970).

Additional characters not included in matrix: available information unspecific or insufficiently detailed, or gradually varying character states

1. Exposure of dorsal cervix and pronotum: (0) not covered by occipital region of head; (1) partly covered by occipital region of head; (2) completely covered by occipital region of head. Pronotum exposed in Glossinidae and other groups of Diptera (Hennig, 1973). Partly visible in *Ornithoica* and Hippoboscinae (Bequaert, 1953), and also in Streblidae (Hennig, 1973; Wenzel & Peterson, 1987: figs 113.1-2).
2. Notopleural suture: (0) distinct; (1) reduced. The notopleural suture is distinctly developed in *Ornithoica* but absent in Hippoboscinae (Hennig, 1973).
3. Mesopleural cleft: (0) not closed; (1) closed. Usually closed in flightless species, such as *Melophagus* and Nycteribiidae (Bequaert, 1953).
4. Position of prothoracic spiracle: (0) laterally; (1) dorsolaterally; (2) dorsally. Shifted dorsad and fully exposed in *Crataerina* and other genera of Hippoboscidae, such as, for instance, *Ornithoctona* (Bequaert, 1953: figs 8A, 10; Schlein, 1970). Placed dorsolaterally and not visible from above in *Ornithoica* (Maa, 1966: fig. 1) and also in *Hippobosca equina* (Bequaert, 1953: fig. 1E). The position within the family varies considerably. It is also placed on the dorsal side in *Basilia* (Nycteribiidae) (Nußbaum, 1960: fig. 2), but not in Streblidae (Zeve & Howell, 1963: figs 4, 5), Glossinidae (Schlein, 1970) and other groups of Diptera (Hennig, 1973).
5. Size of prothoracic spiracle 2: (0) not enlarged; (1) distinctly enlarged. Distinctly enlarged in *Crataerina* and some other genera of Hippoboscidae, and also in *Glossina* (Schlein, 1970: fig. 5). Moderately sized or small in *Ornithoica* (Bequaert, 1953: fig. 8A; Schlein, 1970: figs 8, 12, 14), Streblidae (Schlein, 1970: figs 19, 21; Wenzel & Peterson, 1987: fig. 5) and Nycteribiidae (Nußbaum, 1960: fig. 2). The character varies strongly in Hippoboscidae, and the character state polarity is ambivalent.
6. Metathoracic pleural suture: (0) vertical line; (1) bent with a sharp angle. Bent and forming a sharp angle in Hippoboscidae (Bequaert, 1953).
7. Mesofurcasternum: (0) one completed plate; (1) pair of plates. Divided into two plates in Hippoboscidae (Schlein, 1970). The condition in Streblidae and Nycteribiidae is unclear.

DISCUSSION

Phylogenetic implications and character evolution (Fig. 6)

The calyptrate superfamily Hippoboscoidea is characterized by specialized blood-feeding habits and adenotrophic viviparity (Massonat, 1909; Bequaert, 1953; Hennig, 1973). Within the group, Glossinidae have largely maintained a generalized thoracic

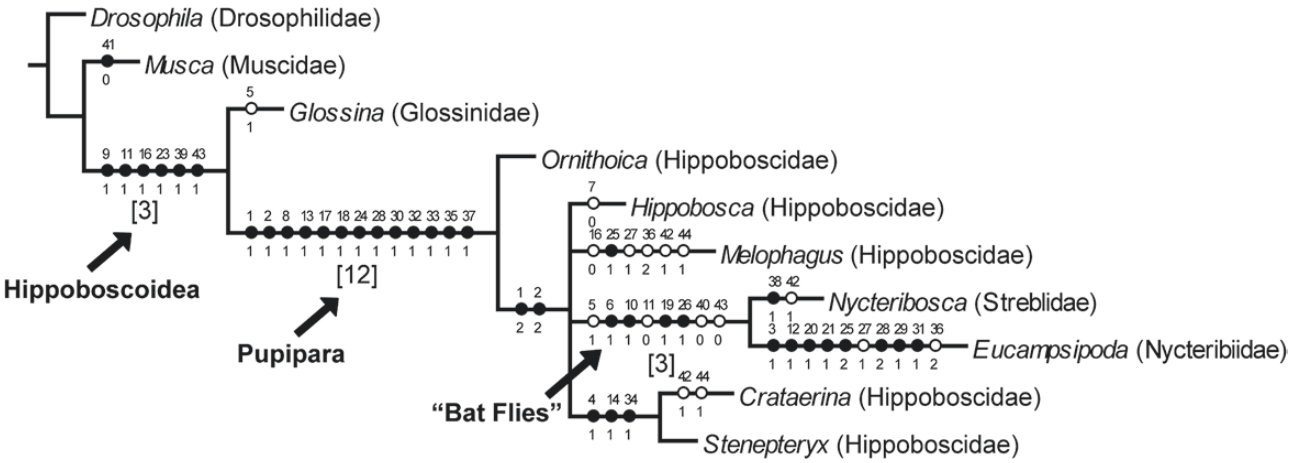

Figure 6. Strict consensus tree of eight equally parsimonious trees (65 steps, Consistency Index = 76, Retention Index = 78) of combined analysis of 44 thoracic morphological data of Hippoboscoidea (NONA, ratchet search, 1000 iterations). Apomorphies are mapped on the tree. The Bremer support values greater than one are labelled under the corresponding branches.

configuration, differing only slightly from the brachyceran groundplan (Schlein, 1970; Hennig, 1973). In contrast, the other families (combined as Pupipara) often display a strikingly modified pattern (Bequaert, 1953; Schlein, 1970). Hippoboscidae, Nycteribiidae and Streblidae are characterized by far-reaching character transformations, apparently often directly linked with specialized ectoparasitism on mammals and birds.

Derived thoracic features shared by Glossinidae and Pupipara are relatively scarce. A potential synapomorphy is the median separation of the procoxae [9: 1] (numbers in square brackets refer to characters and character states; see Fig. 6). However, the condition observed in *Glossina* (Bequaert, 1953: fig. 9B; Schlein, 1970) differs only slightly from what is found in other dipteran groups (Hennig, 1941: pls. 8, 9). Other potential synapomorphies are reduced conditions of both the mesepisternal ridge [16: 1] and mesobasalar apodeme [39: 1], and the ventral fusion of the metepimera [23: 1] (Schlein, 1970).

In contrast to Hippoboscoidea, Pupipara are supported by a multitude of unusual features, with a thoracic pattern very distinctly modified compared with a presumptive groundplan of the superfamily. Conspicuous synapomorphies of the three families are the partial obliteration of the dorsal segmental borders [1: 1] and the distinct flattening of the entire thorax [2: 1], which is about half as high as wide in *Ornithoica*, but usually only one-third in the other groups. In the typical case, the dorsoventral compression of the thorax is linked with a flat or even concave mesopleura (Bequaert, 1953). Derived features of the prothorax are a distinct shortening of the pronotum [8: 1] and the increased size and movability of the procoxa [28: 1]. The mesothorax is apparently the most strongly modified segment. In contrast to Glossinidae and other groups of Diptera, it is strongly broadened [13: 1]. The mesocoxae are widely separated [17: 1], and the mesobasisternum is strongly extended [18: 1]. The movability between the mesotrochanters and the mesofemora is increased [30: 1]. The metacoxae are widely separated, largely immobilized and strongly increased in size [24: 1]. Tarsomeres 2–4 of all legs are shortened (often also tarsomere 1), broadened and flattened [32: 1]. A broad, heel-like claw tooth (Bequaert, 1953: heel; fig. 12K) is present at the base of the strongly developed claws [33: 1]. In contrast to the typical brachyceran condition (Beutel & Gorb, 2001; Friedemann *et al.*, 2014: *Glossina*), the pulvilli are transformed into flexible cylindrical (or apically tapering) structures, with a vestiture of minute hairs (acanthae) [35: 1].

Bat flies, including Streblidae and Nycteribiidae, form a clade in our analyses, as they do in the study by Petersen *et al.* (2007). Potential thoracic synapomorphies of the two families are the loss of the first lateral cervical sclerite [6: 1], a largely reduced probasisternum [10: 1], and the presence of paired metanotal processes projecting into the abdomen [26: 1]. Another complex and unusual feature is a specific deformation of the anterior end of the mesobasisternum [19: 1]; it is folded upwards and backwards, displacing the profurca on a second plane.

The non-monophyly of Streblidae (Petersen *et al.*, 2007) is not addressed here, as we included only one relatively unmodified terminal taxon with available detailed anatomical data (*Nycteribosca*). An unusual derived feature occurring in the family is a club-shaped projection of the third axillary sclerite [38: 1]. In contrast to the other two families, the body shape

varies strongly in Streblidae, with some forms not compressed dorsoventrally but laterally (Wenzel & Peterson, 1987).

Nycteribiidae are characterized by an entire series of character transformations, and most of them are linked with the complete reduction of the flight organs. The wings are completely reduced [36: 2], and the thoracic dorsum is largely membranized [3: 1]. The mesopleural cleft is closed, the mesofurca vestigial [20: 1], and the direct flight muscles are almost completely reduced (see Supporting Information, Appendix 1). The coxae are moved dorsad, similar to the condition in *Melophagus* [27: 1]. Protarsomere 1 is distinctly elongated and curved, at least in the species examined [31: 1]. The mesocoxal ctenidium, which provides additional anchorage in the fur of the bat, is a unique autapomorphy [29: 1]. Cephalic ctenidia have evolved independently within Streblidae, apparently with a similar function (Dick & Patterson, 2007).

It is noteworthy that our analysis did not confirm the monophyly of Hippoboscidae. This result might agree with a recent classification of Diptera (Pape & Thompson, 2018), which includes both Nycteribiidae and Streblidae in this family, replacing the original Pupipara. Interestingly, *Ornithoica*, a genus from Ornithoicinae (Hennig, 1973) or Ornithomyiinae, was placed as sister group of the entire remaining Pupipara, thus rendering the louse flies in the traditional sense indeed paraphyletic. An isolated position of the genus has already been discussed by Hennig (1973), who placed the genus in a separate superfamily (Bequaert, 1953), and also by Maa (1966), who listed an entire series of plesiomorphies of different body regions. In our analysis, apomorphies of Pupipara excluding *Ornithoica* are the far-reaching obliteration of the dorsal segmentation [1: 2] (Bequaert, 1953: less indistinct in *Ornithoica*) and a higher degree of flattening of the thorax [2: 2] (reversal in some Streblidae). Thoracic plesiomorphies preserved in *Ornithoica* include weakly developed humeral calli [14: 0], the presence of a well-developed notopleural suture, the dorsolateral placement of the prothoracic spiracle (also in some other Hippoboscidae), weak lateral thoracic grooves for receiving the profemora, functional wings [36: 0], extensive wing cilia, a large alula, almost complete venation (compared with the other hippoboscid genera), slight dissimilarity in length and shape of the three pairs of legs, and one-toothed claws (Bequaert, 1953; Maa, 1966). Additional plesiomorphies are the completely separated basal antennomere, a short and simple frons, completely developed ocelli, a concealed interantennal area, comparatively well-developed tergal plates (both sexes), a relatively complete sclerotization of the abdominal sternites, and wide host and distributional ranges (Maa, 1966). *Ornithoica* was not included in the sampling of Petersen *et al.* (2007). However, the position of this genus definitely deserves increased attention.

The monophyly of Hippoboscidae excluding *Ornithoica* is not supported in our analysis. Not surprisingly, *Crataerina* is placed as sister taxon of *Stenepteryx*, ectoparasite of the house martin. Both taxa share similar streamlined connection of head and thorax [4: 1], multiple teeth [34: 1], and a similar pattern of wing reduction [36: 1]. Nevertheless, in contrast to Petersen *et al.* (2007), the loss of the capacity for flight may have occurred independently, as suggested by different muscular patterns in the thorax (see Supporting Information, Appendix 1).

Adaptations to ectoparasitism

Adaptations linked to the reproductive mode and larval development were previously described (Meier *et al.*, 1999; Petersen *et al.*, 2007). Transformations of external thoracic characters were discussed by Massonat (1909), Bequaert (1953), Schlein (1970), Maa & Peterson (1987) and in other contributions.

Adults of Pupipara are characterized by a multitude of features more or less closely related to blood feeding, ectoparasitic habits and adenotrophic viviparity (Massonat, 1909; Bequaert, 1953; Maa & Peterson, 1987). Apart from a tendency towards reduction of the flight organs and a compact unit formed by the head and anterior thorax, obvious adaptations are the flattening of the body (Wenzel & Peterson, 1987: exception in some Streblidae) and the rigid connection of the thoracic segments, with the segmental borders obliterated on the dorsal side (Massonat, 1909). Combined with a resilient, 'leathery cuticle' (Bequaert, 1953), this renders the flies very resistant against mechanical damage resulting from defensive movements of the hosts. What requires further investigation, however, is how the ectoparasites prevent injury of internal organs when their body is strongly squeezed.

A side effect of the compaction of the thorax is a posterior shift of dorsoventral muscles of the prothorax. Their mesonotal origin in Pupipara is an unusual feature probably also linked with the reduced size of the pronotum. A feature characteristic of the family Hippoboscidae is the dorsal shift (and large size) of the prothoracic spiracles (Bequaert, 1953; Schlein, 1970). This is likely to be correlated with the widening and flattening of the mesothorax. It is possible that the exposed position improves the ventilation of the middle thoracic region of louse flies. However, this is not a general feature of Pupipara. Moderately sized prothoracic spiracles are present in *Ornithoica* and in other members of the group. Spiracles reduced in size may be correlated with a strongly reduced flight apparatus.

A flightless lifestyle requires less energy and therefore a lower supply of oxygen.

The legs are strongly developed compared with the other groups of Diptera (Bonhag, 1949; Smart, 1959; Ulrich, 1971, 1984; Owen, 1977; Fabian *et al.*, 2016). They are affected in various ways by ectoparasitic habits. Dufour (1845) observed that hippoboscids are able to walk efficiently backwards and laterally without raising their flattened body from the substrate. This ability is presumably linked with the rather freely suspended procoxae, and meso- and metatrochanters with high degrees of freedom. The modifications of the legs apparently enhance the attachment and movability on the hairy or plumed integument of the warm-blooded vertebrate hosts. A conspicuous feature, which is not found in other groups of flies, is the wide separation of the coxal insertions. This probably improves the grasp on the hosts. It reaches a culminating point in the sheep ked *Melophagus* and in nycteribiid bat flies (Massonat, 1909; Bequaert, 1953); the coxae of these advanced ectoparasites are moved onto the dorsal side of the body, a highly unusual feature in insects. The laterally shifted coxal insertions are apparently correlated with the dorsoventral flattening of the body. Owing to this modified coxal arrangement, it is also possible to shift the origin of coxal and trochanteral muscles. *Melophagus*, which lives submerged in the wool of its ovine host (Massonat, 1909: 'plongé dans la laine de son hôte'), shows an exceptionally high degree of specialization compared with other hippoboscids. The thorax is shortened and very compact. The mesonotal sutures are largely obliterated and the flight organs completely reduced (Bequaert, 1953). According to Bequaert (1953), the sheep ked also reaches a maximal degree of mechanical resilience.

The articulation of the coxae of Pupipara is generally modified compared with other flies. The enlarged procoxae are very movable, whereas the mesocoxae and the enlarged metacoxae are largely or completely immobilized. This lack of coxal movement is compensated for by an increased movability in the trochanteral–femoral joint, at least in the case of the midlegs (Schlein, 1970). This enhanced rotation may be beneficial during the attachment process, because it supports the adjustment of the attachment angle of the pretarsus. The tarsi are generally shortened and broadened. The protarsal attachment structures differ indistinctly from the typical brachyceran pattern (Bauchhenß, 1979; Friedemann *et al.*, 2014), probably because of the necessity for clinging to moveable hosts with feathers or fur. The pulvilli are strongly modified in *C. pallida* and other hippoboscids, flexible, cylindrical and covered with a vestiture of specialized minute tenant hairs. Additionally, a slender empodium is present, partly glabrous but partly covered with very short setae. The strongly developed claws play a major role in attachment and locomotion on the host body surface. They are bifid (longitudinally cleft) in ectoparasites of birds, with the noteworthy exception of the genus *Ornithoica*. Bifid claws combined with an elongated and curved claw tooth (Friedemann *et al.*, 2014) may improve the grasp and movability in the plumage.

Claws showing edges with multiple teeth occur in spiders (Aranae) and ensure an efficient grasp on filamentous structures of their safety threads and orb webs (Gorb & Barth, 1994). This is structurally (and probably functionally) very different from the claw apparatus of bird ectoparasites in Hippoboscidae, where the bifid claws are supported by a curved claw tooth, thus forming a trifid functional unit but without a serrated (or multi-toothed) edge.

Flightlessness

Despite profound modifications linked with anatomical dipterism, the pterothoracic flight apparatus of true flies is a highly complex functional unit, as in other groups of Pterygota (Brodsky, 1994; Deora, Gundiah & Sane, 2017). Boettiger & Furshpan (1952) and Boettiger (1957) observed and described the dipteran flight mechanism for the first time in detail using *Sarcophaga bullata* Parker, 1916 (Sarcophagidae). After that, Pringle (1957) proposed a hypothetical three-dimensional model, which was later evaluated and demonstrated with a mechanical model designed by Pfau (2008). A synchronous wing receptor was described by Miyan & Ewing (1984), wing base articulations and the muscular system by Miyan & Ewing (1985), direct flight muscular modulation by Tu & Dickinson (1994, 1996), and the skeletomuscular system of wingless mutants by Fabian *et al.* (2016). The function of the haltere as a mechanosensory control organ was treated by Chapman (1982), wingbeat-synchronous feedback by Fayyazuddin & Dickinson (1999), and passive mechanical coordination by Deora, Singh & Sane (2015). Recently, Deora *et al.* (2017) summarized various structures of the dipteran flight apparatus in detail. The evolutionary pattern of wing reduction in Hippoboscoidea was addressed in a phylogenetic study based on molecular data (Petersen *et al.*, 2007).

Well-developed flight organs are doubtless part of the groundplan of Hippoboscoidea and Pupipara. However, as outlined by Massonat (1909), Bequaert (1953), Schlein (1970) and more recent contributions (Maa & Peterson, 1987; Wenzel & Peterson, 1987), various patterns of reduction occur within the group. In Hippoboscidae, wings can be partly (e.g. *Craterina*, *Stenepteryx*) or completely reduced, they can be shed shortly after emergence or after reaching a suitable

host (e.g. some species of *Hippobosca*), or represented by a solid subcylindrical knob, obviously unsuitable for flight (Maa & Peterson, 1987). A strong tendency towards flightlessness characterizes the Streblidae (Schlein, 1970; Wenzel & Peterson, 1987), and the development culminates in Nycteribiidae. All known species of this family have lost their wings. The flight muscles are almost completely reduced (see Supporting Information, Appendix S1). The thoracic dorsum is largely membranous and the entire body strongly flattened (Nußbaum, 1960; Wenzel & Peterson, 1987).

The flight capacity of *C. pallida* is minimal if not lost completely. In laboratory experiments, the flies were able to perform only small jumps and to glide for very short distances in the air (Eichler, 1939; Büttiker, 1944). Like species of other groups of pterygote insects, *C. pallida* and other hippoboscids are subject to a trade-off between obvious advantages of flight (e.g. efficient escape mechanism, dispersal) and adaptations to a specific ecological condition of a single host; in the case of Pupipara, ectoparasitism (Roff, 1990; Wagner & Liebherr, 1992). Important subcomponents of the flight apparatus of *C. pallida* are reduced or non-functional. The mesothoracic wing base sclerites are indistinct and partly merged with the membranous notopleural region, similar to a condition observed in flightless females of the Japanese winter moth *Nyssiodes lefuarius* (Erschoff, 1872) (Liu *et al.*, 2017). In pterygote insects with a functional flight apparatus, they form an efficient articulation with a click mechanism to transmit force from the thoracic exoskeleton to the wings (Brodsky, 1994; Pfau, 2008; Deora *et al.*, 2017). This mechanism is apparently not possible with the vestigial articulation of *C. pallida*. Another strongly modified sclerotized structure is the mesothoracic pleural wing process, which forms the wing fulcrum on the ventral side of the wing base in insects capable of flight (Brodsky, 1994). In dipterans with a normally developed flight apparatus, the mesopleural wing process is a prominent elongate rod-shaped structure (Bonhag, 1949; Smart, 1959; Schlein, 1970; Ulrich, 1971, 1984). In contrast, it is preserved as only a tiny process on the dorsal mesepimeral margin in *C. pallida*.

The dorsal longitudinal muscle IIdlm1 of dipterans is usually divided into several large bundles connected with the extensive anterior area of the mesonotum (Maki, 1938; Bonhag, 1949; Smart, 1959; Christophers, 1960; Mickoleit, 1962; Ulrich, 1971, 1984; Owen, 1977; Fabian *et al.*, 2016). This is also the case in hippoboscid species with preserved flight capacity (Schlein, 1970). In contrast, in *C. pallida* the origin of this indirect flight muscle is shifted backwards to the posterior mesonotal region. The muscle is distinctly shortened compared with the usual condition in dipterans and composed of a narrow single bundle. From its size and position, it is clearly unsuitable for providing the initial contraction of the segment, which is necessary for wing movements (Brodsky, 1994; Pfau, 2008; Deora *et al.*, 2017). The absence of the dorsoventral muscles II/IIIdvm1, 4 and 5 is obviously related to flightlessness. IIdvm1 is especially reduced in all studied flightless polyneopteran and holometabolan species (Friedrich & Beutel, 2010b; Wipfler *et al.*, 2014; Liu *et al.*, 2017). As indirect wing levators (Brodsky, 1994; Pfau, 2008; Deora *et al.*, 2017), these three muscles are almost generally present in dipterans with a functional flight apparatus (see Supporting Information, Appendix S1). Meanwhile, with the loss of several flight muscles, more space in the thoracic lumen becomes available for enlarged extrinsic leg muscles, including the dorsoventral muscles Idvm15, 18 and 19 and the sterno-coxal muscles Iscm1–4, 6, IIscm1–3, 6 and IIIscm1–3, 6 (Fig. 5). It is conceivable that this enhances the capacity of the legs to attach on the integument of the host.

The mesothoracic direct flight muscles are scarcely affected by the loss of the capacity for flight. Only the tergo-pleural muscle IItpm2 and sterno-pleural muscle IIspm1 are absent, compared with most other dipterans capable of flight (see Supporting Information, Appendix S1). The tergo-pleural muscles were defined as synchronous steering muscles that control higher-order wing kinematic patterns, such as a gear-shift mechanism (Pfau, 2008; Deora *et al.*, 2017). The sterno-pleural muscle IIspm1 is absent in most studied flightless insects (Friedrich & Beutel, 2010b; Wipfler *et al.*, 2014). It probably functions primarily as an indirect wing levator (Friedrich & Beutel, 2008), but may have secondary functions, such as adduction and pronation during wing strokes (Brodsky, 1994). Although the dipteran metathorax is distinctly reduced, the halteres play an important role as a gyroscope mechanism during flight (Deora *et al.*, 2017). Some muscles connected to these structures support flight manoeuvres (Fabian *et al.*, 2016). In *C. pallida*, the absence of the tergo-pleural muscle IIItpm11 is likely to be linked with flightlessness. This muscle is present in species of Hippoboscidae capable of flight (see Supporting Information, Appendix S1).

Some flight-related muscles preserved in *C. pallida* apparently have more than one function (Brodsky, 1994). Several tergo-pleural muscles fulfil the additional function of stabilizing the dorsolateral region (Kozlov, 1986; Brodsky, 1994). The sterno-pleural muscle IIspm2 provides a part of the elastic energy required for the click mechanism at the wing base, and it is also involved in gear shifting during flight (Pfau, 2008). It also stabilizes the relative positions of the mesopleuron and mesofurca, which form an integrated endoskeletal complex (Kozlov, 1986).

Some partly or completely preserved structures of the flight apparatus of *C. pallida* are potential evolutionary relicts (Liu *et al.*, 2017). This applies to the indistinct but present wing base sclerites, a mesopleural wing process that is only partly reduced, and some retained pterothoracic muscles. Conversely, some flight-related muscles can be absent in dipterans with a functional flight apparatus, such as, for instance, the dorsoventral indirect wing levator muscle IIdvm1, which is lacking in *Drosophila*, or the dorsoventral muscle IIdvm7, which is missing in species of Hippoboscidae capable of flight (see Supporting Information, Appendix S1). Their absence is likely to be compensated for by other elements of the skeletomuscular system. This phenomenon was observed in species of Orthoptera and Blattodea (Brodsky, 1994) and recently also in whirligig beetles with a strongly simplified but functional flight apparatus (Liu, Wipfler & Beutel, 2018).

ACKNOWLEDGEMENTS

We thank Arndt Wellbrock, Klaudia Witte (University of Siegen, Germany) and Nils Kreuter (Kiel University, Germany) for providing specimens of *C. pallida*. We also thank Dr M. Jäch and P. Sehnal, curators of the Naturhistorisches Museum Wien, for providing the opportunity to examine other species of Hippoboscoidea. We are grateful to two anonymous reviewers for contructive comments that helped to improve the manuscript. This project was financially supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 642241.

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SUPPLEMENTARY INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Diptera muscular homology chart. Muscle presence is represented by '+' in green, absence by '–' in pink, and uncertainty by '?' or '/' in yellow.

3.4. Study IV

Liu, S.-P., Richter, A., Stößel, A. & Beutel, R.G. (2019)

The mesosomal anatomy of *Myrmecia nigrocincta* workers and evolutionary transformations in Formicidae (Hymenoptera). *Arthropod Systematics & Phylogeny*, (accepted).

Abstract

This manuscript describes the external and internal thoracic structures of swift lousefly *Myrmecia nigrocincta* (Hymenoptera: Formicidae) in detail, with a series of traditional and advanced morphological techniques. More morphological details including fine surface structures, complex configuration of sclerites and internal soft parts can be documented in a short time with the advanced techniques, such as microphotography, scanning electron microscopy (SEM) and computer based 3D reconstruction. Myrmeciinae retain a number of plesiomorphic thoracic features. However, several other subfamilies were placed close to the root of ants in recent molecular and fossil researches. The modified thoracic structures of worker refer to an enlarged prothorax with elongate procoxae and strongly developed muscles of forelegs, highly differentiated legs with cleaning and attachment devices, a reduced flight apparatus with greatly simplified pterothoracic musculature and mechanically reinforced exoskeleton, specialized muscles inserted on the base of the gaster. These specialized thoracic structures assist the ant workers to transport item efficiently, move on various surfaces, protect against predators and increase the defensive capacity.

3.4.1. Reviewed Version

The thoracic anatomy of *Myrmecia nigrocincta* workers and evolutionary transformations in Formicidae (Hymenoptera)

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Abstract

The thoracic skeletomuscular system of workers of *Myrmecia nigrocincta* was examined. A broad spectrum of methods was used, for instance micro-computed tomography combined with computer-based 3D reconstruction. An optimized combination of advanced techniques can not only accelerate the acquisition of high quality anatomical data, but also facilitate a very detailed documentation and visualization. This includes fine surface details, complex configurations of sclerites and also internal soft parts for instance muscles with their precise insertion sites. Myrmeciinae have arguably retained a number of plesiomorphic thoracic features, even though recent molecular phylogenies do not place them close to the root of ants. Our mapping analyses based on previous morphological studies and recent phylogenies revealed few thoracic apomorphies linking formicid subgroups. Out of only four retrieved autapomorphies for the family, three are missing in Myrmeciinae. Nevertheless, it is apparent that profound thoracic transformations took place in the early evolution of ants, especially in the flightless workers. The modified thorax is characterized by four character complexes: **a)** an enlarged prothorax with elongate procoxae, modified coxo-trochanteral articulations, a large plate-like pronotum, strongly developed muscles of the forelegs and especially of the neck region; **b)** highly differentiated legs with complex cleaning and attachment devices; **c)** a reduced flight apparatus with greatly simplified pterothoracic musculature and mechanically reinforced exoskeleton and **d)** strongly developed specialized muscles inserted on the base of the gaster. Structural modifications of the prothorax and neck region allow ant workers to transport items efficiently with a highly movable head with strongly developed cervical muscles. Their differentiated legs enable them to move efficiently on various surfaces and to maintain their complex apparatus of sensilla. The mechanically reinforced thorax provides protection against predators and likely against detrimental environmental agents. The enhanced movability of the gaster increases the defensive capacity with a sting or other mechanisms.

Keywords

Myrmecia, Formicidae, ants, thorax, phylogeny, evolution.

1. Introduction

Even though most species of Formicidae are small and inconspicuous insects, the group is exceptionally popular and also generally known outside the community of entomologists. The diversity of the family is relatively modest with ca. 12 800 described species (BOUDINOT 2015). However, the enormous biomass, the obviously high impact in many ecosystems, elaborate forms of eusociality and complex behavior patterns have attracted intensive attention of researchers since the early days of scientific entomology (see e.g. HÖLLDOBLER & WILSON 1990). Morphological research on Formicidae goes back to the late 19th century (e.g. NASSONOV 1889). Nevertheless, considering the enormous popularity and importance of the group, the available anatomical data are surprisingly scarce. Most morphological investigations were restricted to external skeletal features, whereas detailed and well-documented anatomical studies are still scattered and limited in their taxonomic scope.

A very early study providing anatomical data on "ants, bees and wasps" was presented by LUBBOCK (1881). The homology of external thoracic structures of ants was discussed by NASSONOFF (1889) and JANET (1898). EMERY (1900) homologized structures in different ant castes, and his nomenclature was used by later researchers. SNODGRASS (1910a) briefly described the thorax of an ant worker in a comprehensive study on Hymenoptera. TULLOCH (1935) compared external thoracic structures of alates and workers. The anatomy and life history of workers of *Camponotus herculeanus pennsylvanicus* De Geer, 1773 were described by FORBES (1938) and the anatomy of *Rhytidopenera metallica* (Smith, 1858) by WHELDEN (1960). Workers from seven subfamilies were described by REID (1941) for a general comparison of wingless and short-winged types in Hymenoptera. MARKL (1966) and SAINI et al. (1982) described the thoracic skeletomuscular system of single species, the former aso covering the thoracic nervous system. The tracheal system was investigated by KEISTER (1962). DE GUSMÃO et al. (2001), BILLEN et al. (2011) and BILLEN (2017) examined the metapleural gland under morphological and functional aspects.

Many studies on external structures were used in a taxonomic context (e.g. WILSON et al. 1967; GOTWALD & KUPIEC 1975; GOTWALD & SCHAEFER 1982; BOLTON 2003; BOUDINOT 2015), partly also covering fossil taxa (e.g. GRIMALDI et al. 1997; ENGEL & GRMALDI 2005; BARDEN & GRIMALDI 2012). BROWN (1954) presented the first comprehensive morphology based phylogenetic study. He did not explicitly use a Hennigian (or cladistics) approach in this contribution. Nevertheless, due to his very extensive taxonomic work and profound knowledge of the group, his tentative evolutionary tree is almost consistent with results of recent analyses of molecular data (KELLER 2011). Using scanning electronic microscopy, BARONI URBANI et al. (1992) and KELLER (2011) acquired more detailed external morphological data to retrieve the phylogeny of Formicidae. Combining anatomical

data, phylogenetic systematics and locomotor function, KELLER et al. (2014) analyzed the thoracic evolution in ant castes and trade-off between different behavioral patterns.

Recently, computer-based 3D reconstruction was used to increase the efficiency and accuracy of the documentation of external and internal characters, and also to facilitate sharing complex morphological data (FRIEDRICH et al. 2013; WIPFLER et al. 2016). HITA GARCIA et al. (2017) applied these methods in descriptions of two new species from Madagascar. However, fine details like muscle insertions were not documented in that study, and generally the use of this approach (and other innovative methods) is still limited in studies on Formicidae and related groups. Consequently, our primary aim was to provide a detailed documentation of the thorax of an ant worker using a broad spectrum of techniques, including microphotography, scanning electron microscopy (SEM), micro-computed tomography (μCT) and 3D reconstruction. For our investigation we chose a species from the subfamily Myrmeciinae. Even though this group is likely not close to the root of the family, it shows a maximum number of discernable thoracic sclerites, and it therefore possibly close to the primitive thoracic status of Formicidae (WARD & BRADY 2003). We homologize the thoracic muscles observed in *Myrmecia* with those previously described for species of other groups, notably the honeybee (SNODGRASS 1942) but also other representative of Aculeata and more "ancestral" taxa like Xyelidae and Tenthredinidae (VILHELMSEN 2000a; b; MAKI 1938). We compare our observations with conditions found in formicid alates and members of other groups of Aculeata. Finally, we present some interpretations on the functional and evolutionary background of modifications occurring in different castes of ants.

2. Materials and methods

2.1. Specimens examined

Myrmecia nigrocincta Smith, 1858, worker, preserved in 70% ethanol, collected by R. Jordan in Australia, Queensland, Mount Hypipamee on September 2, 2002 in an open forest habitat. The species identification follows the key from OGATA & TAYLOR (1991).

2.2. Hand drawings

One specimen was manually dissected in 70% ethanol under a Zeiss Stemi SV11 with an additional Euromex Illuminator EK-1 lighting system. The thoracic sclerites were drawn with full lines, margins below other sclerites with dotted lines. The legs were omitted, except the coxal elements. The figures were drawn with pencil under the microscope, scanned into the computer and finished with Adobe Illustrator CC.

2.3. Computer-based 3D reconstruction

One specimen was dehydrated in an ethanol series (from 70% to 100%) transferred into Acetone and dried at the critical point (EmiTech K850 Critical Point Dryer). It was scanned in a SkyScan221 micro-CT (FSU Jena) with beam strength of 40 kV and 320 µA. In a 360° Scan pictures were taken every 0.2° with an exposure time of 150 ms. A pixel size of 0.9 µm was achieved. The thoracic segments of the specimen were reconstructed three-dimensionally using FEI Amira 6.0 based on the µCT-image stack.

2.4. Microscopic photography and scanning electronic microscopy

One specimen was air dried after fixing it in position over night in 100% ethanol. It was photographed with a Keyence VH-Z20R to record the general body shape and coloration. Another specimen after the critical point drying was coated with gold (EmiTech K500 sputter coater). Micrographs were taken with Philips XL 30 ESEM (FEI) and ResAlta Scandium software.

2.5. Reconstruction of character evolution

External characters of the thorax for the mapping analysis were adopted from previous morphology-based phylogenetic studies including No. 10–15 from BARONI URBANI et al. (1992) and No. 49–94 from KELLER (2011). These two references provide comprehensive data on the external skeletal morphology. We excluded character No. 10–12 from BARONI URBANI et al. (1992) that are duplicated as characters No. 49, 60 and 62 in KELLER (2011). Based on parsimony inference we assigned the most plausible character state to the groundplan of each subfamily if it was represented by more than one terminal with variation in this feature (NINOMIYA & YOSHIZAWA 2009). In cases of ambiguity we scored multiple states for the terminal. We then checked uninformative characters in WinClada with the function "Mop uninformative chars" and deleted them. Finally, 30 characters (Table S1) for 19 formicid subfamilies and 3 outgroups Scolidae, Bradynobaenidae and Vespidae were mapped on a manually reconstructed tree in WinClada with the function "Move branch mode", using the phylogenomic topology from MOREAU & BELL (2013). It contains a large number of terminal taxa, extensive gene regions and relatively unambiguous alignments. The phylogenetic pattern was also confirmed in a recent comprehensive study of BLANCHARD & MOREAU (2017).

We also homologized the thoracic muscles of 12 species of Hymenoptera with those observed in our studied species *M. nigrocincta* (Tables 1; S2). The muscular characters come from LUBBOCK (1881), MAKI (1938), DUNCAN (1939), SAINI et al. (1982), VIHELMSEN (2000a; b), MIKÓ et al. (2007), SNODGRASS (1942) and ALAM (1951). The information from the last two references was extracted from "TABLE XXII" of MATSUDA (1970). All of them refer to 7 families including Xyelidae, Tenthredinidae, Ichneumonidae, Braconidae, Scelionidae, Vespidae, Apidae and

Formicidae. Among them, Vespidae is represented by three species of *Vespa*; and Formicinae from Formicidae by three species: two of them containing the information from both alate gynes and flightless workers (LUBBOCK 1881; SAINI et al. 1982), and one only contains the information from the worker (MARKL 1966).

2.6. Terminology

The terminology for the thoracic extroskeleton is based on KELLER (2011), and on FRIEDRICH & BEUTEL (2008a) for internal skeletal structures and muscles.

A – posterolateral margins of pronotum; Ar – arolium; B – meso-metapleural suture; Btar1/2/3 – pro-/meso-/metabasitarsus; BtarS – basitarsus setae; Ca – calcar; CaBa – brush on anterior side of calcar; CaLa – lamella of calcar; CaPe – pectinate-shaped structure of calcar; Cl – claw; Cx1/2/3 – pro-/meso-/metacoxa; Fe – femur; Fe1/2/3 – pro-/meso-/metafemur; Fu1/2/3 – pro-/meso-/metafurca; Hm – hairy membrane, ISp – propodeal spirable; IT – propodeum; Lcv – lateral cervical sclerites; Ma – manubrium; N1/2/3 – pro-/meso-/metanotum; Pl – planta; Pl1/2/3 – pro-/meso-/metapleuron; Pl3G – metapleural gland; Ses – setae stout; Sp2/3 – meso-/metathoracic spiracle; SpL – spiracle lobe; StrC – strigil comb, StrN – strigil notch; Tar – tarsus; Tar1/2/3 – pro-/meso-/metatarsus; Tb – tibia; Tb1/2/3 – pro-/meso-/metatibia; Tr1/2/3 – pro-/meso-/metatrochanter; TspA2/3 – meso-/metatibia antero-dorsal apical setae; TspP2/3 – meso-/metatibia posterior apical setae.

Abbreviations for muscles are based on the terminology of FRIEDRICH & BEUTEL (2008a). Two newly introduced abbreviations are used, IA1 for the 1st elevator of the abdomen, IA2 for the 2nd elevator (LUBBOCK 1881).

3. Results

3.1. Skeletal structures

3.1.1. General appearance

The entire tagma appears very slender in dorsal view and greatly simplified in its entire skeletal configuration. It is densely covered by minute setae (Fig. 3). In lateral view the pro- and mesothorax form an arch from the strongly narrowed cervical region to the mesocoxal insertion area. The propodeum appears parallelogram-shaped in lateral view, resting like a saddle on the posterior mesothorax. The strongly slanting and very distinct meso-metapleural suture (B: Figs. 2A; 3C; 4A) extends from the metathoracic spiracle to the lateral mesocoxal articulation.

3.1.2. Cervical region and prothoracic skeleton

The segment appears elongated and pear-shaped in dorsal view, and slightly curved in lateral view, with an evenly curved, ascending pronotum and a concave ventral side. The cuticle is slightly rugose, without any conspicuous surface modifications.

The cervical membrane connects the strongly narrowed foramen occipitale with the narrow anterior prothoracic margin. The wide and concave surface of the occipital region of the head appears very large in comparison to the anterior prothorax. The neck region lacks exposed sclerotized cervicalia, but an invaginated internal process represents a modified lateral cervical sclerite (Lcv: Fig. 4D).

The elongate pronotum (N1: Figs. 1A, B; 2A–C; 3A, C; 4A) is evenly rounded along its lateral margins; it reaches its greatest width at its posterior 1/4 and very distinctly narrows before it connects with the mesonotum, with evenly rounded posterolateral edges (A: Figs. 2A; 3C; 4A); the anterior margin is slightly bent upwards; it is distinctly narrowed, about 1/3 as wide as the maximum width of the segment; the lateral edge of the pronotum is rounded; the posterior edge appears more or less concave depending on the angle of view; a distinctly reduced prophragma is present as a sclerotized edge at the hind margin of the pronotum. The large propleuron (Pl1: Figs. 1B, C; 2A, C; 3B, C; 4A) is bent downwards, thus covering the lateral part of the segment and also the entire ventral area, where both parts meet along a ventromedian suture; a distinct, evenly curved suture separates it from the pronotum, running almost exactly parallel to the lateral segmental border; antero-laterally it forms paired roughly triangular projections, enclosing a V-shaped incision, which forms the propleural antero-ventral edge of the segment.

The large, strongly elongated procoxae (Cx1: Figs. 1B, C; 2A, C; 3B, C; 4A) extends postero-ventrad from the posterior propleural margin; they are inserted in transversely oval procoxal cavities. Antero-medially the profurca (Fu1: Fig. 4B) arises from a rhombic sclerite between the procoxae, the only exposed sternal element; an extensive process for muscles attachment arises from the anterior side of the apical part of the profurca.

3.1.3. Mesothoracic skeleton

The mesonotum (N2: Figs. 1A, C; 2B, C; 3A, C; 4A) is a completely undivided, moderately large, oval sclerite; it is about 1.5 as long as wide and evenly rounded on all sides; antero-laterally it bears a pair of mesothoracic spiracles (Sp2: Figs. 2A; 3C; 4A), which are covered by semicircular spiracle lobes (Spl: Figs. 2A; 3C; 4A). The large mesopleuron (Pl2: Figs. 1B, C; 2A, C; 3B, C; 4A) is dorsally connected with the lateral mesonotal margin; it expands over the entire lateral and ventral areas; ventro-medially it invaginates into the thoracic lumen to form a long triangular discrimen, which is posteriorly connected to the mesofurcal base; antero-ventrally it articulates with the posterior procoxal base. The cone-shaped mesocoxae are distinctly shorter than the procoxae and appear directed outwards in their resting position (Cx2: Figs. 1B, C; 2A, C; 3A–C; 4A); they are inserted at the postero-ventral margin of the

mesopleura; they are almost adjacent medially, even though the internal openings of the mesocoxal cavities are widely separated and distinctly smaller than their prothoracic counterparts. The narrow mesofurca (Fu2: Fig. 4B) is curved in lateral view; it originates from the middle area between the paired mesocoxae; from there it extends antero-dorsad and connects to the dorsal part of the mesopleuron; a slender antero-median mesofurcal process serves as attachment area of a ventral longitudinal muscle.

3.1.4. Skeleton of metathorax and propodeum

The metanotum (N3: Figs. 2A, B; 3A) is a very narrow and undivided element between the annular metathoracic spiracles (Sp3: Figs. 2A, B; 3A, C; 4A), which are close to the postero-lateral mesonotal margin. The very large propodeum (IT: Figs. 1A, C; 2A, B, C; 3A, C; 4A) anteriorly connects with the metanotum and mesopleura, and ventrally with the small metapleura (Pl3: Figs. 2A, C; 3B; 4A). A sclerotized discrimen extends from the ventral midline between the paired metapleural halves upward into the thoracic lumen. Dorso-laterally a pair of slit shaped spiracles are present (ISp: Figs. 2A, B; 3C; 4A), and postero-laterally the paired opening of the flocculent metapleural glands (PlG3: Figs. 2A; 3C; 4A, E), close to a sclerotized process linked with the following abdominal segment. A weak carina is present antero-dorsad of the gland opening. The metacoxae (Cx3: Figs. 1B, C; 2A; 3A–C; 4A) are similar to the mesocoxae in shape and size, but posteriorly oriented in their resting position; they are inserted at the posterior edge of the metapleura; the internal openings of the metacoxal cavities are medially connected, thus forming a broad, transverse postero-ventral aperture. The narrow metafurca (Fu3: Fig. 4B) originates from the median area between the metacoxae and extend antero-dorsad along the anterior discrimen; anteriorly they connect the postero-dorsal part of mesofurca; this part forms a semi-cylindrical structure that ensheathes the very long and slim ventral longitudinal muscle.

3.1.5. Legs

All three pairs of legs are long and slender and covered by a short pubescence. Additionally fine long setae are dispersed over the entire surface, with the length of the hairs decreasing distally. The foreleg is the shortest and the hindleg the longest. However, the procoxa (Cx1: Figs. 1B, C; 5A) is distinctly elongated, almost twice as long as its meso- (Cx2: Figs. 1B, C; 5B) and metathoracic counterparts (Cx3: Figs. 1B, C; 5C). All trochanters (Tr1–3: Figs. 5A–C) are short, barrel-shaped sclerites. The protrochanter connects laterodistally with the procoxa, with an articulation allowing rotatory movements; the meso- and metatrochanters connect distally with their respective coxae with a hinge-like articulation, resulting in a more restricted movability. The femur (Fe1–3: Figs. 1A–C; 5A–C), the longest element of all three

legs, is slightly bulbous in its proximal half, especially in the fore- and hindlegs, and narrows distally. All femuro-tibial articulations are hinge-like (Fig. 5A). The tibiae (Tb1–3: Figs. 1A–C; 5A) are rather straight and narrower than the femur. All tarsi (Tar1–3: Figs. 1A–C; 5A) are five-segmented; the basitarsus is about as long as the remaining tarsomeres combined; tarsomere 4 is the shortest and bilobed; tarsomere 5 bears paired claws and an arolium (Fig. 5A).

A complex armature is present on the distal parts of all three legs, very complex on the foreleg and slightly less on the hindleg. The protibia bears a row of four stout setae distally on its posteroventral surface; the posterior side bears three stout setae apically near the calcar (Ca: Figs. 5E, F) of the strigil (KELLER 2011: antenna cleaner), which articulates on the ventral side of the protibia. The calcar carries a lamella (CaLa: Fig. 5F) proximally and a pectinate-shaped structure (CaPe: Fig. 5F) on its distal region; on its anterior surface it bears a brush of clubbed microtrichia, running parallel to the lamellate and pectinate edge and covering most of its surface on this side; densely packed, short, spine-like microtrichia are present on the ventral surface of the calcar. The probasitarsus (Btar1: Figs. 5E, F) forms the corresponding part of the strigil; its anterior surface bears paddle-shaped setae similar to the clubbed microtrichia of the calcar; very similar setae are also inserted distally on the antero-ventral protibial surface. The probasitarsus bears shorter, apically rounded setae on its posterior and ventral side; the comb of the strigil (StrC: Fig. 5F) is inserted in a proximal notch of the probasitarsus (StrN: Fig. 5F), and a row of stout setae (Ses: Fig. 5F) extends parallel to it on the posterior surface of this tarsomere; more widely spaced stout setae are continuous with this row and reach the distal end of the probasitarsus. The meso- and metatibia also bear stout apical setae, antero-dorsally and posteriorly on the midleg and posteriorly on the hindleg; additionally, both carry two spurs distally on the ventral side (Figs. 5G, H). The mesotibial spurs (TspA2, TspP2: Fig. 5G) are very similar to each other in length and configuration, with short, spine-like microtrichia on the ventral side and a row of cuticular teeth on the dorsal edge. The metatibial spurs (TspA3; TspP3: Fig. 5H) show some resemblance to the strigil; while the smaller anterior spur is similar to its mesotibial counterpart, the larger posterior spur carries a comb of microtrichia on its dorsal side, quite similar to that of the calcar; additionally, this spur bears a small brush of club-like microtrichia on its posterior side, smaller than the one on the anterior side of the calcar but otherwise similar; paddle-shaped setae are inserted on the postero-ventral metabasitarsus, similar to those of the anterior probasitarsal surface. A group of blunt setae is inserted distally on the postero-ventral metatibial surface. The meso- and metabasitarsi bear lateral rows of stout setae like the probasitarsus, additionally a sulcus is present on their anterodorsal surface.

3.1.6. Pretarsal structures

The claws (Cl: Figs. 6A–C) of all legs are well-developed. They bear an apically rounded preapical tooth that is broader than the apical part. A vestiture of short setae is present on the proximal two thirds of the claws, three long setae are inserted ventro-laterally, and a field of minute hairs is present on the ventral base. The arolium (Ar: Fig. 6) and its supporting sclerites originate between the claws; the bone-shaped elongate manubrium is dorsally attached to tarsomere 5; it bears several small setae proximally and two larger setae proximad its middle region; membranous areas with minute spine-like protuberances are present laterad the manubrium (Ma: Figs. 6B, D). The surface structure at the lateral membranous bases of the arolium is similar, whereas its ventral and lateral regions are smooth. The deeply concave dorsal surface resembles a wicker basket, with scale-like microtrichia on the edges, most of which carry a single microtrichium on the tip. Ventrally, the planta (Pl: Fig. 6A) is densely covered with setae. The unguitractor plate is not visible on the images presented here and the arcus is also concealed (FEDERLE et al. 2010)

3.2. Musculature (Fig. 4)

3.2.1. Prothoracic muscles

Dorsal longitudinal muscles

Idlm1, M. prophragma-occipitalis, long and slender, slightly wider in its middle region; O (=original): median part of posterior pronotal margin, I (=insertion): dorsally on posterior edge of occipital region, close to midline.

Idlm5, M. pronoto-phragmalis anterior, fan-shaped, strongly narrowing anteriorly, narrower posteriorly; O: postero-lateral area of pronotum; I: latero-median part of posterior pronotal margin.

Dorsoventral muscles

Idvm5, M. pronoto-cervicalis anterior, broad muscle, narrowing towards insertion site on lateral cervical sclerite; O: antero-lateral area of pronotum; I: lateral cervical sclerite.

Idvm9, M. profurca-occipitalis, long slender bundle; O: anterior area of profurca; I: dorsal occipital region.

Idvm18, M. pronoto-coxalis lateralis; O: postero-lateral area of pronotum; I: lateral procoxal rim.

Tergo-pleural muscles

Itpm1, M. pleurocrista-occipitalis, very large muscle, narrowing towards insertion on occipital region; O: postero-lateral area of proepimeron; I: dorso-median area of occipital region.

Itpm2, M. propleuro-occipitalis, very large muscle, larger on propleuron, narrowing towards insertion on occipital region; O: postero-dorsal area of propleuron; I: dorso-median area of occipital region.
Itpm4, M. pronoto-apodemalis anterior; O: ventro-lateral area of pronotum; I: dorsal area of profurca.
Itpm5, M. pronoto-apodemalis posterior; O: postero-lateral area of pronotum; I: profurcal anterior process.
Itpm6, M. pronoto-intersegmentalis; O: postero-ventral area of pronotum; I: basal area of profurca.

Pleuro-coxal muscles
Ipcm4, M. propleuro-coxalis superior, triangular, narrowing towards insertion on procoxal rim; O: postero-dorsal area of proepimeron; I: lateral procoxal rim.
Ipcm8, M. propleuro-trochanteralis, long muscle; O: postero-dorsal area of proepimeron; I: protrochanteral tendon.

Ventral longitudinal muscles
Ivlm1, M. profurca-cervicalis, slender muscle; O: profurcal anterior process; I: lateral cervical sclerite.
Ivlm3, M. profurca-tentorialis, long slender bundle; O: anterior area of profurca; I: ventral occipital region.
Ivlm7, M. profurca-mesofurcalis, two subcomponents, the median bundle slender and shorter, the lateral one widening towards mesofurcal insertion site; O: posterior area of profurca; I: median bundle on anterior mesofurcal process, lateral subunit on antero-dorsal part of mesofurca.

Sterno-coxal muscles
Iscm1, M. profurca-coxalis anterior; O: postero-ventral area of proepimeron close to the basal part of profurca; I: anterior procoxal rim.
Iscm3, M. profurca-coxalis medialis, wider on profurca, narrowing towards insertion on procoxal rim; O: dorsal part of profurca; I: mesally on procoxal rim.
Iscm4, M. profurca-coxalis lateralis, triangular muscle, wider on profurca, narrowing towards insertion on procoxal rim; O: dorsal part of profurca; I: laterally on procoxal rim.
Iscm6, M. profurca-trochanteralis, long muscle; O: dorsal part of profurca; I: protrochanteral tendon.

3.2.2. Mesothoracic muscles

Dorsoventral muscle

IIdvm5, M. mesonoto-coxalis posterior, very large muscle, narrowing towards insertion on mesocoxal rim; O: posterior margin of mesonotum; I: laterally on mesocoxal rim.

Ventral longitudinal muscle

IIvlm7, M. mesofurca-abdominosternalis, very long and slender; O: postero-dorsal part of mesofurca; I: anterior margin of abdominal segment II.

Sterno-coxal muscles

IIscm1, M. mesofurca-coxalis anterior, very large triangular muscle, narrowing towards insertion on mesocoxal rim; O: ventral area of mesopleuron; I: anteriorly on mesocoxal rim.

IIscm3, M. mesofurca-coxalis medialis, triangular muscle, narrowing towards mesocoxal rim; O: basal part of mesofurca; I: mesocoxal rim.

IIscm4, M. mesofurca-coxalis lateralis, very large triangular muscle, narrowing towards on mesocoxal rim; O: ventral area of mesopleuron; I: laterally on mesocoxal rim.

3.2.3. Muscles of metathorax and propodeum

Dorsoventral muscle

IIIdvm4, M. metanoto-coxalis anterior, large muscle, narrowing towards metacoxal rim; O: posterior margin of metanotum; I: antero-laterally on metacoxal rim.

IIIdvm5, M. metanoto-coxalis posterior, very large muscle, narrowing towards metacoxal rim; O: antero-lateral area of propodeum; I: postero-lateral metacoxal rim.

Ventral longitudinal muscle

IIIvlm2, M. metafurca-abdominosternalis, slender muscle; O: postero-ventral part of metafurca; I: anterior margin of abdominal second segment.

Sterno-coxal muscles

IIIscm1, M. metafurca-coxalis anterior, large triangular muscle, narrowing towards metacoxal rim; O: ventral area of metafurca; I: laterally on metacoxal rim.

IIIscm4, M. metafurca-coxalis posterior; O: postero-ventral area of metafurca; I: laterally on metacoxal rim.

Muscles of propodeum

IA1, 1st elevator of abdomen, long feather-shaped muscle; O: dorso-medial area of propodeum; I: antero-dorsal margin of abdominal segment II.

IA2, 2nd elevator of abdomen; O: dorso-lateral area of propodeum; I: antero-ventral margin of abdominal segment II.

4. Discussion

4.1. Morphological techniques

The anatomical investigations in this study were carried out with only five specimens. Nevertheless, the workflow and combination of different techniques allowed a very detailed documentation of skeletal structures and also internal soft parts. Combinations of traditional and modern techniques can greatly facilitate the acquisition of detailed morphological data. Traditional anatomic studies can be of great value and highly accurate and detailed (e.g. DUNCAN 1939; MARKL 1966; SAINI et al. 1982; MIKÓ et al. 2007). However, information on coloration and 3-dimensional effects are usually limited or lacking (e.g. MAKI 1938). Complex anatomical illustration usually requires great experience and outstanding drawing skills (e.g. LUBBOCK 1881; WEBER 1969; see ALLGAIER 2008). Besides this, especially in the case of small or very small objects, structural details may be easily overlooked without high quality microtome sections or micro-CT data. In the case of the thorax this may concern minute muscles of the wing base, which are generally difficult to observed based only on dissections.

Microphotography accurately records important characters including coloration, degree of sclerotization and transparency of cuticle (WIPFLER et al. 2016). Accompanying the verbal description, microphotographs provide a more intuitive impression of habitus and configuration of an insect specimen (Fig. 1). They are very well suited for gradual changes of color and texture of the cuticle, which are very difficult to reproduce with traditional drawing techniques.

Scanning electron microscopy (SEM) provides precise information on fine surface structures (FRIEDRICH et al. 2014; WIPFLER et al. 2016). Compared with hand drawn figures (Fig. 2), SEM micrographs very clearly depicted minute microtrichia or scales with stereo effect and also fine wrinkles or other patterns of microsculpture (Fig. 3). High magnification and resolution allows documenting fine details not accessible with other methods (Figs. 5E–H; 6: microtrichia and tiny hairs on the legs and claws), allowing their interpretation in a functional and evolutionary context. Handling of specimens and working with limited material is facilitated by a recently developed rotatable specimen holder (POHL 2010). Additional advantages are reduced charging of surfaces and a homogenous black background.

Microcomputed tomography (µ-CT) can greatly accelerate the acquisition of anatomical data and is less cost intensive than histological sectioning (WIPLFER et al. 2016). High quality µ-CT data are an ideal basis for 3D reconstruction, which has arguably triggered a renaissance of insect anatomy in the last 15 years (FRIEDRICH & BEUTEL 2008b; FRIEDRICH et al. 2014; WIPFLER et al. 2016). With µ-CT and suitable reconstruction (e.g. Amira, VGStudio) in an optimized workflow, external and internal structures such as sclerites, muscles, nerves and gland can be very clearly visualized (Fig. 4; WIPFLER et al. 2016). The description of the new species *Terataner*

balrog Hita Garcia from Madagascar was based on this method (HITA GARCIA et al. 2017: fig. 11). The entire study is an exemplary contribution in taxonomy, even though fine details like muscle insertions or nerves were not identified separately.

A systematic morphological database was established by YODER et al. (2010: Hymenoptera Anatomy Ontology) for the megadiverse Hymenoptera (WIPFLER et al. 2016). Websites such as antbase.org and antweb. org (AGOSTI 2005) provide numerous highly quality images of Formicidae with standardized terminology. The enormous potential of these anatomical data collections in a taxonomic and phylogenetic context was emphasized by WIPFLER et al. (2016). The technical procedures and the workflow presented in this study may contribute to the optimization of these ventures in the future.

4.2. Phylogenetic patterns and the "ancestral status" of Myrmeciinae

In a study on the rediscovered Australian genus *Nothomyrmecia* TAYLOR (1978) characterized Myrmeciinae as "the most structurally generalized ants" and their behavior as "significantly primitive" (also see WARD & BRADY 2003). In fact, the subfamily has arguably retained an entire series of plesiomorphic features. This includes six maxillary and four labial palpomeres in all castes, 12 antennal segments in females and 13 in males, paired calcariae on the middle and hind tibiae, tarsal claws with a strongly developed median tooth, a furcula and two-jointed gonostyli associated with the stinging apparatus, and an active damming mechanism of the proventriculus of workers (TAYLOR 1978). In an unpublished phylogenetic tree from Brown's laboratory (around 1986), Myrmeciinae were placed close to the root, conform with an apparent proximity to the groundplan of Formicidae (Keller 2011). Meanwhile, phylogenetic concepts changed (e.g. RABELING et al. 2008; MOREAU & BELL 2013; BLANCHARD & MOREAU 2017). Moreover, new ant fossils were discovered recently, likely belonging to the stemgroup of the family (e.g. GRIMALDI et al. 1997; ENGEL & GRIMALDI 2005; PERRICHOT et al. 2008; BARDEN & GRIMALDI 2012). They provide new morphological data, which are relevant in the context of ancestral features and the early evolution of the group. Therefore, the "ancestral status" of Myrmeciinae and the groundplan of Formicidae deserve further discussion.

The monophyly of Formicidae is consistently supported in analyses based on morphological (BARONI URABNI 1989; BARONI URBANI et al. 1992; KELLER 2011) or molecular data (MOREAU et al. 2006; BRADY et al. 2006; RABELING et al. 2008; MOREAU & BELL 2013). In contrast, the branching pattern within the group is affected by changing phylogenetic approaches, with different phylogenies suggested by morphological characters and different molecular data sets. BARONI URBANI (1989) and BARONI URBANI et al. (1992) placed Formicinae as an early branch in studies based on morphology (BARONI URBANI 1989: fig. 2; 1992: fig. 5), suggesting that some generalized formicid features were conserved in this subfamily. The analyses of extensive molecular data with a broad taxon sampling (MOREAU & BELL 2013;

BLANCHARD & MOREAU 2017) placed the small and specialized subfamilies Leptenillidae and Martialinae as the first two branches in a tree of extant ant taxa, followed by a "ponerine complex" (e.g. Ponerinae, Amblyoninae), and then Myrmeciinae + Pseudomyrmeciinae in a large clade with nine other subfamilies, among them Dolichoderinae and Ecitoninae. This lineage is placed as sister taxon of a clade comprising Ectatomminae + Heteroponerinae, and the two diverse subfamilies Formicinae and Myrmicinae. In re-analyses of morphological data with fossil taxa included, a basal position of the extinct Sphecomyrmine in Formicidae s.l. (including stemgroup taxa) was supported (GRIMALDI et al. 1997). Maintained plesiomorphies of this group are the presence of two mandibular teeth and an antenna with a short scape and long, flexible funiculus (DLUSSKY 1983; GRIMALDI et al. 1997).

Recently phylogenetic results (MOREAU & BELL 2013; BLANCHARD & MOREAU 2017) clearly show that Myrmeciinae are not a "basal" group of ants, but in fact separated by seven nodes from the root of the tree (MOREAU & BELL 2013: fig. 4). However, this does not exclude that the subfamily has preserved more ancestral features than most other formicid subgroups, as pointed out by TAYLOR (1978). In contrast to Myrmeciinae, the "basally" placed Martialinae are highly specialized, with an array of apomorphies linked with hypogaeous, and predacious and predacious habits, aside from some preserved plesiomorphic ant features (RABELING et al. 2008).

Our mapping analyses (Fig. 7) using the phylogeny of MOREAU & BELL (2013) yielded only four apomorphies of Formicidae. The presence of metapleural glands [10.1] is very likely a derived groundplan feature (BARONI URBANI 1989). It strengthens the social community with anteseptics or antibiotic substances (BARONI URBANI 1989; BILLEN et al. 2011; BILLEN 2017). However, its absence in males of several species implies a limited role in cooperative activities (BARONI URBANI 1989). The propodeal spiracle without bulla [13.1], a round to oval atrial opening [14.1] and the absence of stout setae on the posterior protibial apex [17.0] were also retrieved as autapomorphies. The noteworthy absence of all three features in Myrmeciinae is rated as a result of reversals in our mapping analysis. A clade Myrmeciinae + Pseudomyrmeciinae is supported by three possible thoracic synapomorphies, a calcar of the strigil with a basal lamella [19.1], propraetarsal claws with a preapical tooth [24.1] and the presence of a metabasitarsal sulcus [30.1]. However, apparent homoplasy in these characters makes the polarity assessment ambiguous. This also applies to the open metacoxal cavity [16.0], which was retrieved as an autapomorphy of Myrmeciinae. It cannot be excluded that some presumptive results of reversals are in fact retained plesiomorphies in Myrmeciinae, with parallel evolution of derived conditions in several formicid subgroups. Similar selective pressure may have resulted in a pattern, which is less parsimonious than secondary loss in Myrmeciinae.

4.3. Thoracic groundplan of Formicidae

The differentiation into flightless workers and sexual morphs with preserved flight organs is a crucial groundplan apomorphy and key innovation of Formicidae (HÖLLDOBLER & WILSON 1990).

Compared with a generalized pattern of Aculeata (SNODGRASS 1910b; 1925: Apidae; DUNCAN 1939: Vespidae), alate sexual morphs of ants show only limited modifications of the thorax, such as for instance wings with a modified venation, or obsolete mesopleural ridges. The pronotum is larger than the short and clasp-shaped structure of Vespidae and Apidae (SNODGRASS 1910b; 1925; DUNCAN 1939), but distinctly shorter than in workers. The propleuron, a triangular plate-like structure, articulates with its lower posterior angle with the coxa, and with the occipital region with its anterior margin. Cervicalia are fused with the propleura like in the groundplan of Hymenoptera postulated by VILHELMSEN (2000a). The small but separate sclerites of the neck region identified in the honeybee by SNODGRASS (1925) are likely due to reversal. Like in more generalized aculeatan groups like Vespidae and Apidae, a functional wing articulation is preserved in the pterothoracic segments of alate ants, with axillary sclerites (SAINI et al. 1982), notal wing process and mesothoracic basalar and subalar sclerites as attachment sites of direct flight muscles.

The highly modified thorax of workers, documented in detail for *Myrmecia* in the present study, is part of a complex of groundplan apomorphies. The pronotum is a solid and extensive plate-like structure. The propleura cover the entire lateral and ventral prothoracic regions and meet ventromedially, with only a small exposed prosternal sclerite between the procoxae. A strongly pronounced notopleural edge forms an articulation with the posterolateral head capsule. The cervical sclerite is completely fused with the internal skeleton of the propleuron. The procoxa is distinctly enlarged relative to the meso- and metacoxa in workers of *Myrmecia* and other groups, including stemgroup fossils (WILSON et al. 1967). This is arguably an additional groundplan apomorphy of Formicidae, with parallel evolution in other aculeate groups such as Dryinidae (Chrysopoidea), which catch prey or hosts for their larvae with the forelegs (WALOFF 1974).

A crucial character complex is the complete loss of wings and flight related structures (Figs. 2; 3). The mesonotum (N2: Figs. 1A, C; 2A–C; 3A, C; 4A) forms a single sclerite without separate elements like prescutum, scutum or scutellum, and also without mesophragma (Fig. 4B). The mesopleuron (Pl2: Figs. 1B, C; 2A, C; 3B, C; 4A) is also undivided, and like in the prothorax its paired halves are fused ventromedially. Axillary sclerites, notal wing processes, basalare and subalare are missing in both pterothoracic segments of workers (Figs. 2A; 3A, C; 4A). The metanotum (N3: Figs. 2A, B; 3A) is very narrow and undivided. The metapleuron (Pl3: 2A, C; 3B; 4A) is strongly reduced. The elongated metafurca (Fu3: Fig. 4B) connects anteriorly with the slender mesofurca (Fu2: Fig. 4B) and encloses the very slender ventral longitudinal muscle (IIvlm7: Fig. 4C).

Compared with members of other families of Hymenoptera (Tables 1; S2), the prothoracic musculature appears largely unmodified in alate and flightless ant castes.

The dorsal longitudinal muscles Idlm1 and 5, the dorsoventral muscles Idvm5, 9 and 18, the tergo-pleural muscles Itpm1–5, the pleuro-coxal muscles Ipcm1, 4 and 8, the ventral longitudinal muscle Ivlm1, 3 and 7 and sterno-coxal muscles Iscm1, 2, 4 and 6 are present in workers of *Myrmecia* and very likely also in the groundplan of Formicidae. As plesiomorphic features alate queens retain a relatively complete muscle set in the mesothorax. This includes well-developed dorsal longitudinal bundles and also dorsoventral, tergo-pleural and pleuro-coxal muscles (LUBBOCK 1881; SAINI et al. 1982). Among them the presence of muscles IIdlm1, IIdvm1 and 6 and IItpm7 and 9 is likely ancestral for Formicidae. In contrast, workers, including those of *Myrmecia*, have lost most mesothoracic muscles, except those inserted on the mesocoxal rim and mesotrochanter. The comparison of queens and workers with representatives of other families of Hymenoptera suggests that the sterno-coxal muscles IIscm1–3 and 6 belong to the groundplan of Formicidae. The number of metathoracic muscles of queens is only slightly less than in other families of Hymenoptera (Tables 1; S2). In contrast, the muscle set is greatly simplified in workers.

Our results confirm that thoracic groundplan features differ profoundly between ant castes, with moderate modifications in alate forms and far-reaching transformations in workers. Anatomical investigations of stemgroup ant fossils (e.g. GRIMALDI et al. 1997; ENGEL & GRIMALDI 2005; PERRICHOT et al. 2008; BARDEN & GRIMALDI 2012) using micro-computed tomography should have high priority. Combined with detailed morphological data on related groups, this will not only allow a more reliable assessment of the groundplan, but also a reconstruction of early evolutionary transformations in the group.

4.4. Thoracic modifications in workers and their functional background

The thoracic morphology of ant workers was apparently shaped by different but interrelated phenomena. This includes complete winglessness combined with a mechanically reinforced pterothorax, a very movable head with a strongly developed neck musculature, unusually differentiated legs as efficient cleaning tools and locomotor organs, and a highly movable gaster with a stinging apparatus preserved in *Myrmecia* and in the groundplan.

The loss of wings in ant workers has consequences beyond the loss of the ability to fly. To reduce structures required in the context of flight opens perspectives to specialize in other directions (e.g. BURD 2000; SCHILMAN & ROCES 2005; BOHN et al. 2012). Although workers are “cheaply manufactured” to support the colonial economy (PEETER & ITO 2015), considering them as a wingless version of the alate queens would be an oversimplification (KELLER et al. 2014).

Compared with alate forms, the pronotum of workers is greatly enlarged, apparently in correlation with an enhanced muscular apparatus (KELLER et al. 2014). This increases the movability in the neck region (SNODGRASS 1935; HARTENSTEIN

2006; KELLER et al. 2014) and also enables the ants to lift and carry objects with their head, such as for instance prey, pieces of plants or seed (e.g. GORB & GORB 1999; MOLL et al. 2010; KELLER et al. 2014; NGUYEN et al. 2014).

An important modification of workers is the far-reaching fusion of sclerites. The dorsal pterothoracic sclerites are reduced to undivided and undifferentiated notal elements. Notal wing processes and axillary sclerites, important elements controlling flight movements (BRODSKY 1994), are dispensable in flightless morphs and therefore reduced, resulting in an increased mechanical rigidity of the segments. The pterothoracic sclerites are largely fused in ant workers (e.g. RICHARDS 1956; KELLER 2011). This leads to a mechanically very compact thorax with a minimum of exposed membranous surfaces, a condition also occurring in beetles, even though achieved in a different way and in most cases with a retained functional flight apparatus (e.g. BEUTEL & HAAS 2000). The reinforced thorax enhances mechanical protection against predators on the ground and possibly also increases the barrier against harmful environmental agents. Ant workers are the caste that forages outside the nest and are strongly exposed to these factors throughout their adult live span (HÖLLDOBLER & WILSON 1990; JEMIELITY et al. 2005). This results in selective pressure favoring the formation of rigid exoskeletal structures, made possible mainly by the reduction of the flight apparatus.

In a study on the mesothorax of workers, KELLER et al. (2014) noted the simplification of the mesonotum, the reduction of the mesophragma and the loss of the dorso-longitudinal muscle IIdlm1. This structural complex generally initiates the first wing depression in the flight stroke cycle in pterygote insects with retained flight ability (BRODSKY 1994), but it is usually modified or distinctly reduced in secondarily flightless insects (WIPFLER et al. 2014; LIU et al. 2017). The absence of dorso-ventral muscles II/IIIdvm1 is another common feature related to the loss of the flight capacity (KOZLOV 1986; WIPFLER et al. 2014; LIU et al. 2017). As depressors of the notum during wing elevation (BRODSKY 1994), they are dispensable in flightless forms, and in fact completely absent in all examined ant workers (Tables 1; S2).

Some flight related muscles are retained in worker ants, such as the dorsoventral muscles II/IIIdvm4, 5 and 7, which are even exceptionally well developed. These elements of the muscular system can fulfill more than one function, as wing levators in forms capable of flight, but also in the context of leg movements (KOZLOV 1986; BRODSKY 1994; LIU et al. 2017).

Linked with the loss of the flight capacity, locomotion on different substrates plays an essential role in ant workers. Consequently the legs are highly differentiated legs (Figs. 1; 5) and well-developed muscles operating leg movements are present in all three thoracic segments. The enlarged, elongated procoxae likely allow more flexible movements of the forelegs, together with a modified coxo-trochanteral articulation. The sterno-procoxal muscles IIscm1 and 4 distinctly are greatly enlarged in workers. This supports efficient movements on the ground including a broad range of specific activities (HÖLLDOBLER & WILSON 1990), such as jumping (CLACK 1951:

7.5–10 cm), digging (WALLIS 1962; SUDD 1969), trophallaxis, antenna cleaning (WALLIS 1962), prey handling (MASUKO 2009), grooming of queens in the case of leaf cutter ants and behaviors related to the hygiene fungus gardens (FERNANDEZ-MARIN et al. 2003).

The differentiated arolia and claws further support efficient movement on various surfaces (FEDERLE et al. 2001), even though well-developed pretarsal attachment devices are a common feature in aculeate hymenopterans (FRANSTEICH & GORB 2004). The unusually complex armature of the distal leg parts, especially in the case of the fore- and hindlegs, also plays a role in different functional contexts, for instance efficient cleaning of the antennae. Perfect functioning of cuticular sensilla on the antennae and other body parts likely plays an essential role for ant workers.

A last essential character complex is the highly movable gaster, with an aculeate singing device retained in the groundplan of the family (HÖLLDOBLER & WILSON 1990). The increased movability of the gaster is supported by a strongly developed and specialized petiole musculature (IA1, IA2: Figs. 4C, D), with positive effects in the context of defense and prey capture (HASHIMOTO 1996). Protection against predators apparently plays an important role for the flightless workers. Aside from the sting, additional defensive adaptations of workers have evolved, including for instance gland secretions or sharp exoskeletal spines (BUSCHINGER & MASCHWITZ 1984; BLANCHARD & MOREAU 2017).

Structural modifications of ant workers can be seen as optimization for different tasks important for the colony. At the same time, the simplified structure of the worker thorax means “less investment” compared with winged eusocial insects with a complex flight apparatus. Highly efficient workers produced at “low coast” are likely one of the main factors contributing to the ecological dominance and success of ants (PEETERS & ITO 2015). In summary, the reduced flight apparatus of ant workers is one side of an evolutionary trade-off: on one hand obvious advantages of flight like for instance dispersal, and on the other various options to optimize other functions in the context of a particularly successful like strategy (ROFF 1990; WAGNER & LIEBHERR 1992).

5. Acknowledgements

We are very grateful to Raul Jordan for the donation of valuable ant material. Prof. Dr. Lars Vilhelmsen (Natural History Museum of Denmark), Dr. Istvan Mikó (Pennsylvania State University) and Dr. Lars Krogmann (Staatliches Museum für Naturkunde Stuttgart) provided valuable literature. This is also gratefully acknowledged. This study reflects only the author’s view and the Research Executive Agency is not responsible for any use that may be made of the information it contains. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 642241.

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Electronic Supplement files:

S1. Combined skeletomuscular characters

S2. Muscular homology chart of Hymenoptera (Present with "+" or muscular name in green, absent with "-" in pink, uncertain with "?" or "/" in yellow. In Formicidae, the muscles only present in workers are labeled in dark green; muscles only occurring in alate castes labeled in dark blue.)

Table 1. Musculatures of Formicidae (SAINI et al. 1982; LUBBOCK 1881; MARKL 1966), Vespidae (DUNCAN 1939) and Apidae (SNODGRASS 1942) (Present with "+" or muscular name in green, absent with "-" in orange, uncertain with "?" or "/" in yellow. In Formicidae, the muscle present only in worker labeled in dark green; only in alate castes labeled in dark blue.)

Family	**Vespidae**	**Apidae**		**Formicidae**		
Genus	*Vespula*	*Apis*	*Myrmecia*	*Camponotus*	*Lasius*	*Formica*
ldlm1	-	40 + 41	+	1	a	40 & 41
ldlm2	-	-	-	-	-	-
ldlm3	-	-	-	-	-	-
ldlm4	-	-	-	-	-	-
ldlm5	lis1[50] & lis2[51]	45	+	-	-	45
ldlm6	-	-	-	-	-	-
ldvm1	-	-	-	-	-	-
ldvm2	-	-	-	-	-	-
ldvm3	-	-	-	-	-	-
ldvm4	-	-	-	-	-	-
ldvm5	lpm1[37] & lpm2[38]	47	+	6	c	46 & 47
ldvm6	-	-	-	-	-	-
ldvm7	-	46	-	-	-	-
ldvm8	-	-	-	-	-	-
ldvm9	Ois2[33]	43	+	2	a1	43
ldvm10	-	-	-	-	-	-
ldvm11	-	-	-	-	-	-
ldvm12	-	-	-	-	-	-
ldvm13	-	-	-	-	-	-
ldvm14	-	-	-	-	-	-
ldvm15	-	-	-	-	-	-
ldvm16	-	-	-	-	-	-
ldvm17	-	-	-	-	-	-
ldvm18	llm6[48]	55	+	-	-	55
ldvm19	-	-	-	-	-	-
ltpm1	-	42b? & 42c?	+	-	b1	42/1
ltpm2	Ois1[32]	42a?	+	-	c1 & c2	42/2
ltpm3	lpm3[39] & lpm4[40]	48	-	-	-	48
ltpm4	lpm5[41]	49	+	10	e	49
ltpm5	lpm6[42]	50	+	-	f	50
ltpm6	-	-	+	-	g	-
lppm1	-	-	-	-	-	-
lppm2	-	-	-	-	-	-
lspm1	-	-	-	-	-	-
lspm2	-	-	-	-	-	-
lspm3	-	-	-	-	-	-
lspm4	-	-	-	-	-	-
lspm5	-	-	-	-	-	-
lspm6	-	-	-	-	-	-
lspm7	-	-	-	-	-	-
lpcm1	llm7[49]	mcr	-	-	d1	mcr
lpcm2	-	-	-	-	-	-
lpcm3	llm5[47]	57	-	-	-	-
lpcm4	-	-	+	-	h	53
lpcm5	llm2[44]	53	-	-	-	-
lpcm6	-	-	-	-	-	-
lpcm7	-	-	-	-	-	-
lpcm8	llm3[45]	61	+	-	k	-
lvlm1	lfp[36]	51	+	-	d	51
lvlm2	-	-	-	-	-	-
lvlm3	Ois3[34] & Ois4[35]	44	+	3	b	44
lvlm4	-	-	-	-	-	-
lvlm5	-	-	-	-	-	-
lvlm6	-	-	-	-	-	-

lvlm7	lis4[53] & lis5[54]?	52	+	18 & 19	m & n	52
lvlm8	-	-	-	-	-	-
lvlm9	-	-	-	-	-	-
lscm1	llm1[43]	54	+	-	i1?	54
lscm2	llm4[46]	56	-	-	i	56
lscm3	-	-	+	-	-	-
lscm4	-	-	+	-	-	-
lscm5	lis3[52]	58	-	-	-	-
lscm6	llm3[45]?	61?	+	-	l	61
lscm7	-	-	-	-	-	-
lldlm1	lldl1[56]	71	-	20	β	-
lldlm2	-	-	-	-	-	-
lldlm3	llis1[72]	70	-	-	-	-
lldvm1	lldv1[57]	72	-	21	θ	-
lldvm2	-	-	-	-	-	-
lldvm3	-	-	-	-	-	-
lldvm4	-	-	-	-	-	-
lldvm5	-	-	+	-	-	-
lldvm6	-	82	-	23	-	-
lldvm7	-	-	-	-	o	-
lldvm8	lldv2[58]	78	-	-	-	-
lldvm9	-	-	-	-	-	-
lltpm1	-	-	-	-	-	-
lltpm2	mut[69]	74	-	-	-	-
lltpm3	-	-	-	-	-	-
lltpm4	-	-	-	-	-	-
lltpm5	llpm4[62]	75	-	-	-	-
lltpm6	-	-	-	25	-	-
lltpm7	m3Ax[63]	76a	-	24a	-	-
lltpm8	-	-	-	-	-	-
lltpm9	llpm2[60] & llpm3[61]	76b & 76c	-	24b	-	-
lltpm10	-	-	-	-	-	-
lltpm11	llpm5[64]	-	-	-	-	-
lltpm12	-	-	-	-	-	-
llppm1	-	-	-	-	-	-
llppm2	-	-	-	-	-	73
llspm1	llpm1[59]	77	-	-	-	-
llspm2	llfpl1[70] & llfpl2[71]	79	-	-	-	-
llspm3	-	-	-	-	-	-
llspm4	-	-	-	-	-	-
llspm5	-	-	-	-	-	-
llspm6	-	-	-	-	-	-
llspm7	-	-	-	-	-	-
llspm8	-	-	-	-	-	-
llpcm1	-	-	-	-	-	-
llpcm2	-	-	-	22	-	-
llpcm3	lllm1[65]	80	-	-	-	-
llpcm4	-	-	-	-	-	-
llpcm5	-	-	-	-	-	-
llpcm6	-	-	-	-	-	-
llvlm1	-	-	-	-	-	-
llvlm2	-	-	-	-	-	-
llvlm3	llis2[73]	-	-	-	-	79?
llvlm4	-	-	-	-	-	-
llvlm5	-	-	-	-	-	-
llvlm6	-	-	-	-	-	-
llvlm7	-	-	+	35	v	118
llscm1	lllm2[66]	81	+	28	p & s	81 & 82
llscm2	lllm4[68]	83	-	-	q	83
llscm3	lllm4[68]?	83?	+	29 & 31	r	83
llscm4	-	-	+	30	t	80
llscm5	-	-	-	-	-	-
llscm6	lllm3a&b[67]	86	-	-	o	86
llscm7	-	-	-	-	-	-
llldlm1	llldl[75]	96	-	-	-	-

IIIdlm2	-	-	-	-	-	-
IIIdlm3	-	-	-	-	-	-
IIIdvm1	-	-	-	-	θ	-
IIIdvm2	-	-	-	-	-	-
IIIdvm3	-	-	-	-	-	-
IIIdvm4	-	-	+	40	y?	103
IIIdvm5	-	-	+	-	y?	105
IIIdvm6	IIIpm5[80]	105	-	37	-	-
IIIdvm7	-	-	-	-	x	-
IIIdvm8	-	-	-	-	-	-
IIItpm1	-	-	-	-	-	-
IIItpm2	-	-	-	-	-	-
IIItpm3	-	-	-	-	-	-
IIItpm4	-	-	-	-	-	-
IIItpm5	IIIpm4[79]	97 & 98 & 99	-	-	-	-
IIItpm6	-	-	-	39	-	-
IIItpm7	IIIpm2[77]	100	-	38a	-	-
IIItpm8	-	-	-	-	-	-
IIItpm9	IIIpm2[77]?	-	-	38b	-	-
IIItpm10	-	-	-	-	-	-
IIItpm11	IIIpm3[78]	102	-	-	-	-
IIItpm12	-	-	-	-	-	-
IIIppm1	-	-	-	-	-	-
IIIppm2	-	-	-	-	-	-
IIIspm1	IIIpm1[76]	101	-	-	-	-
IIIspm2	-	-	-	-	-	-
IIIspm3	-	-	-	-	-	-
IIIspm4	-	-	-	-	-	-
IIIspm5	-	-	-	-	-	-
IIIpcm1	-	-	-	-	-	-
IIIpcm2	-	-	-	36	-	-
IIIpcm3	IIIIm4[84]	103	-	-	-	-
IIIpcm4	-	-	-	-	-	-
IIIpcm5	-	-	-	-	-	-
IIIpcm6	-	-	-	-	-	-
IIIpcm7	-	-	-	-	-	-
IIIvlm1	-	-	-	-	-	-
IIIvlm2	IIIis2[86]?	118	+	33	u1	119
IIIvlm3	-	-	-	-	-	-
IIIscm1	IIIIm1[81]	104	+	41	z	104 & 105
IIIscm2	IIIIm2[82]	106	-	-	x1	106
IIIscm3	IIIIm2[82]?	106?	-	43	z1	106
IIIscm4	-	-	+	42	y1	103
IIIscm5	-	-	-	-	-	-
IIIscm6	IIIIm3[83]	109	-	-	x	109
IA1	Iadl1[88]	120	+	34	u	120
IA2	Iadl2[89]	119	+	32	w	121

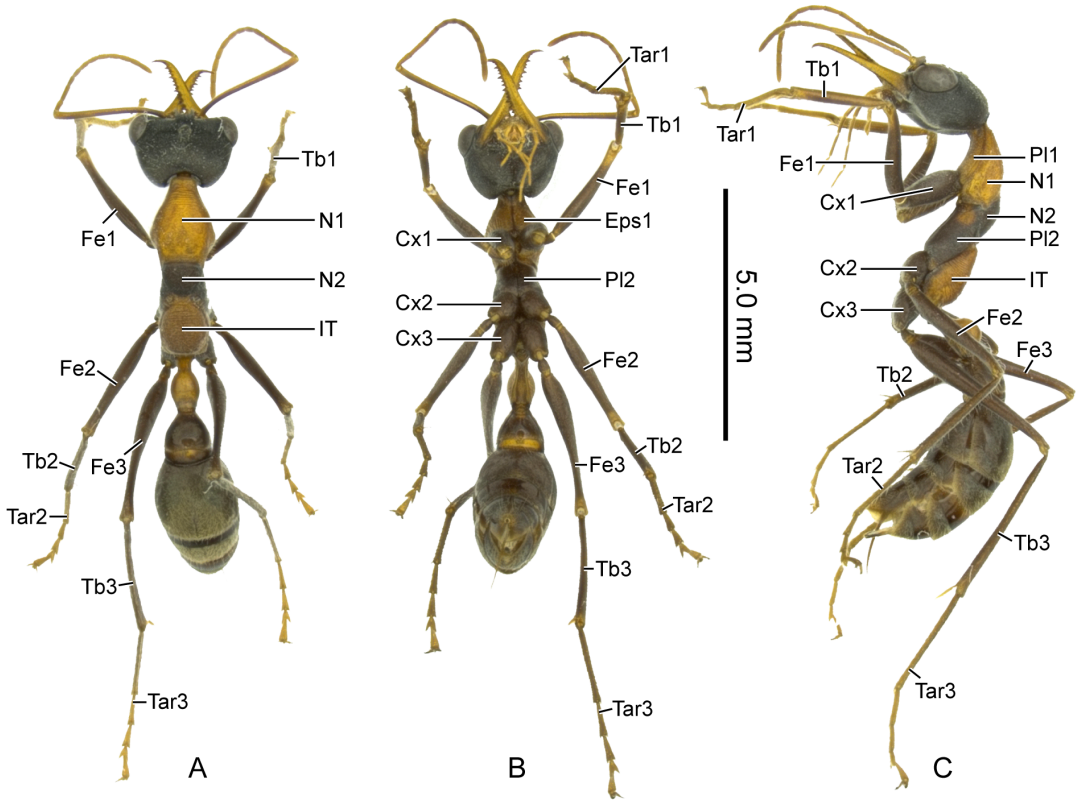

Fig. 1. *Myrmecia nigrocincta*, habitus, digital photographs. **A**: dorsal view, **B**: ventral view, **C**: lateral view.

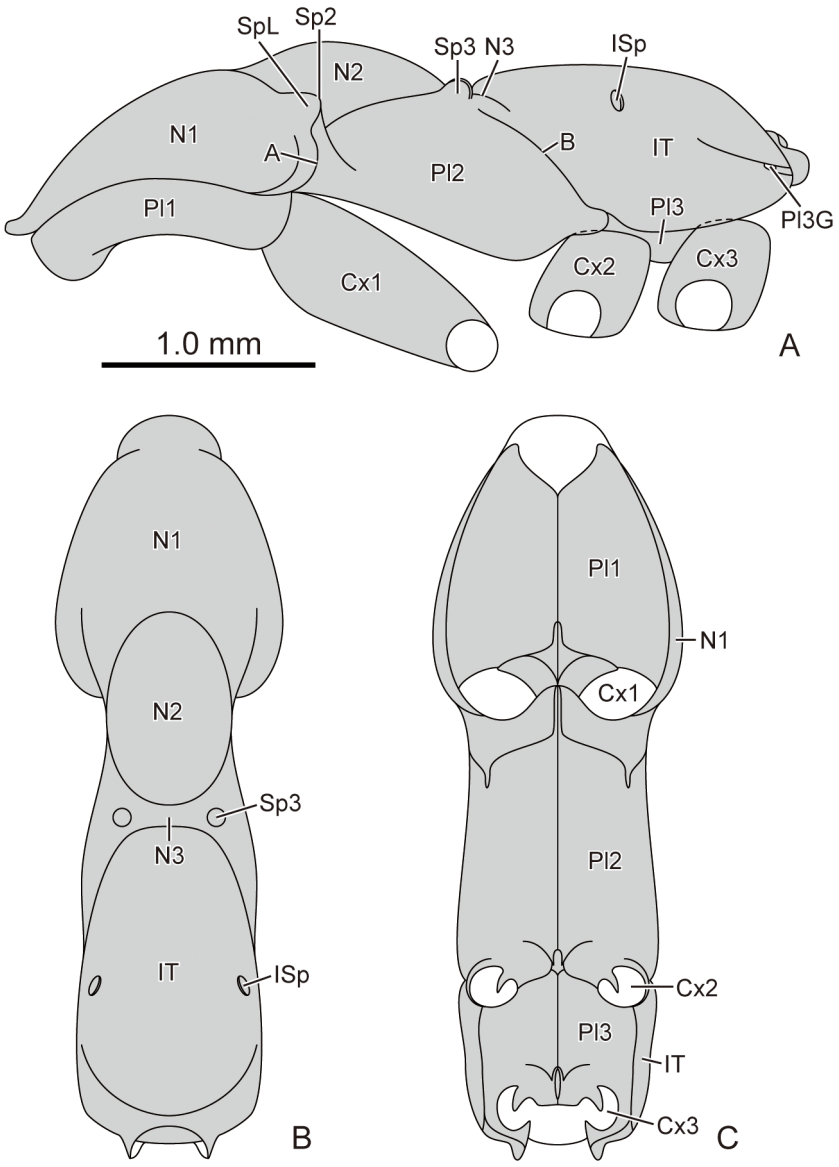

Fig. 2. *Myrmecia nigrocincta*, thoracic exoskeleton, line drawings. **A**: lateral view, **B**: dorsal view, **C**: ventral view.

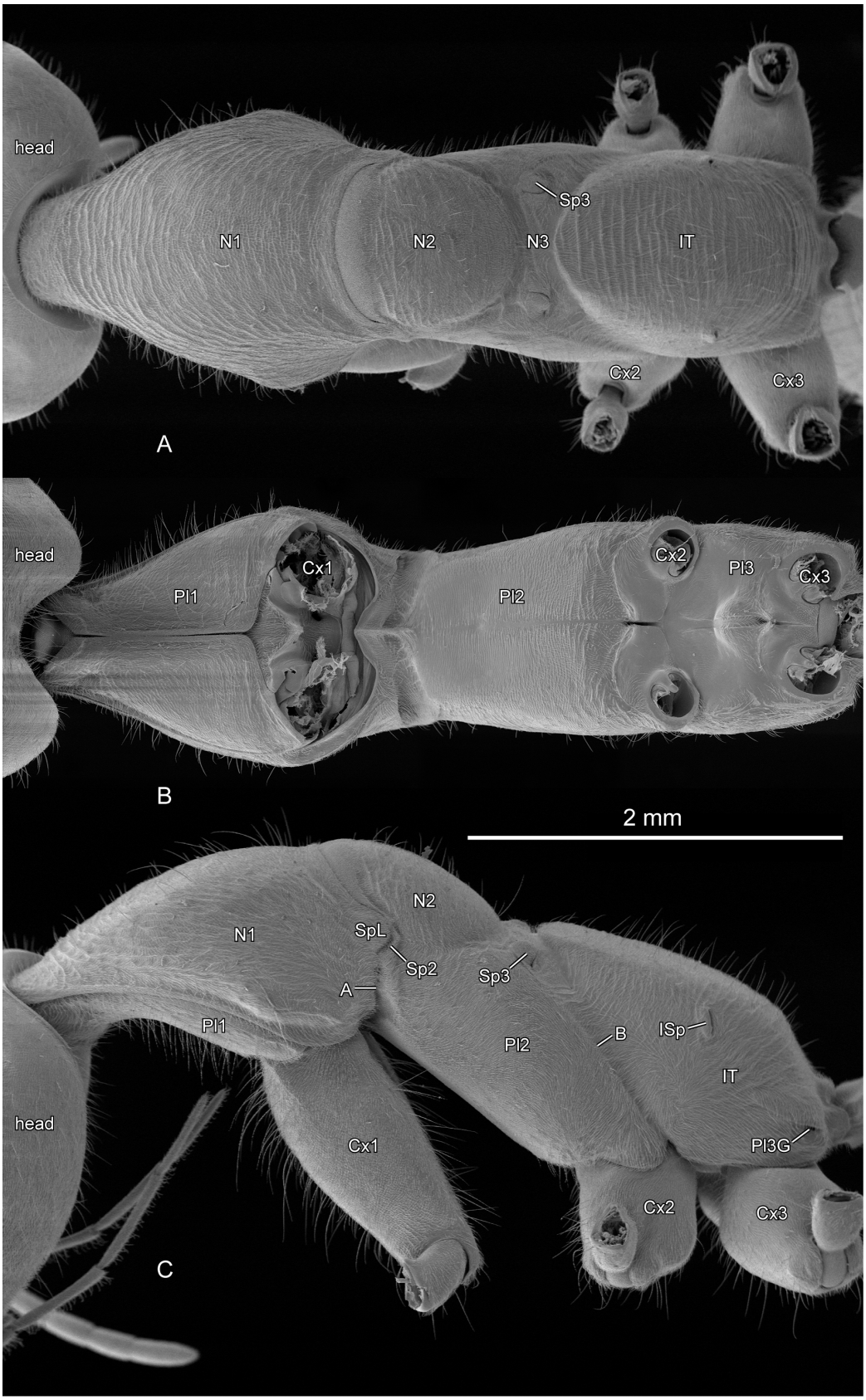

Fig. 3. *Myrmecia nigrocincta*, thoracic exoskeleton, SEM micrographs. **A**: dorsal view, **B**: ventral view, **C**: lateral view.

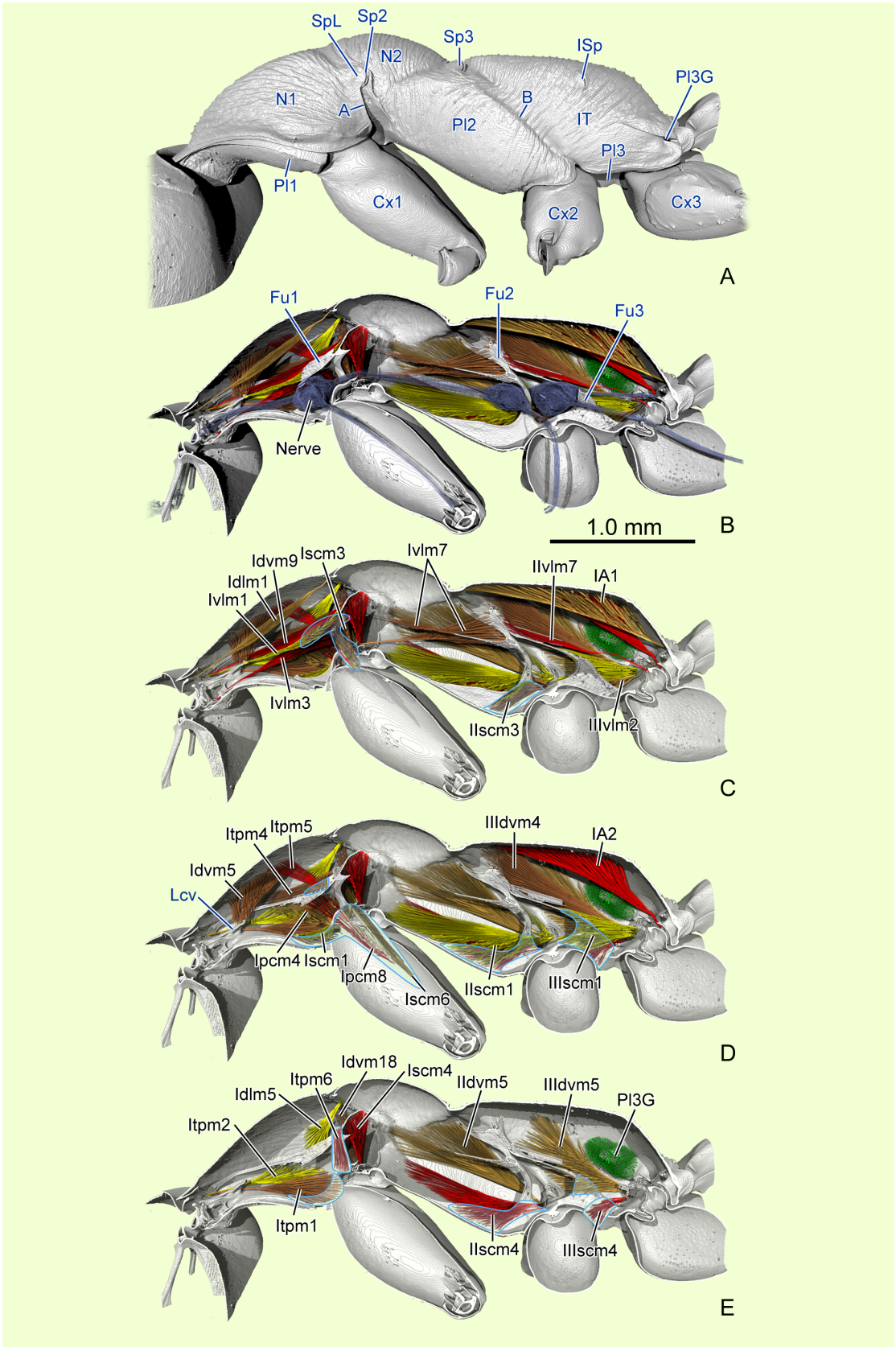

Fig. 4. *Myrmecia nigrocincta*, thorax, 3D reconstruction. **A**: lateral view of exoskeleton, **B**–**E**: endoskeleton, muscles removed layer by layer.

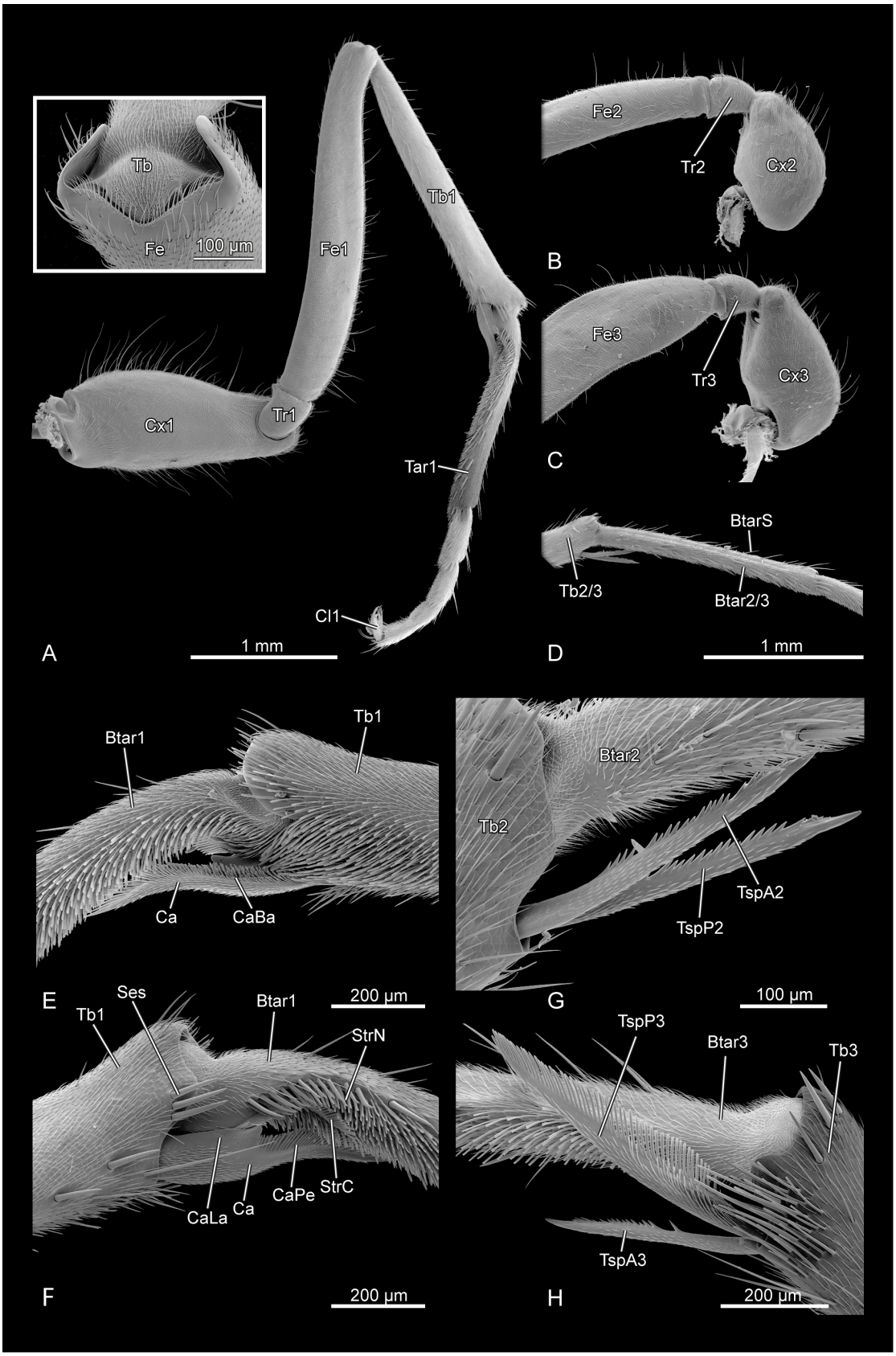

Fig. 5. *Myrmecia nigrocincta*, legs, SEM micrographs. **A**: proleg, **B**: midleg, **C**: hindleg, **D**: articulation between meso-/metatibia and meso-/metabasitarsus; **E**: strigil; **G**: mesotibial spurs; **H**: arolium.

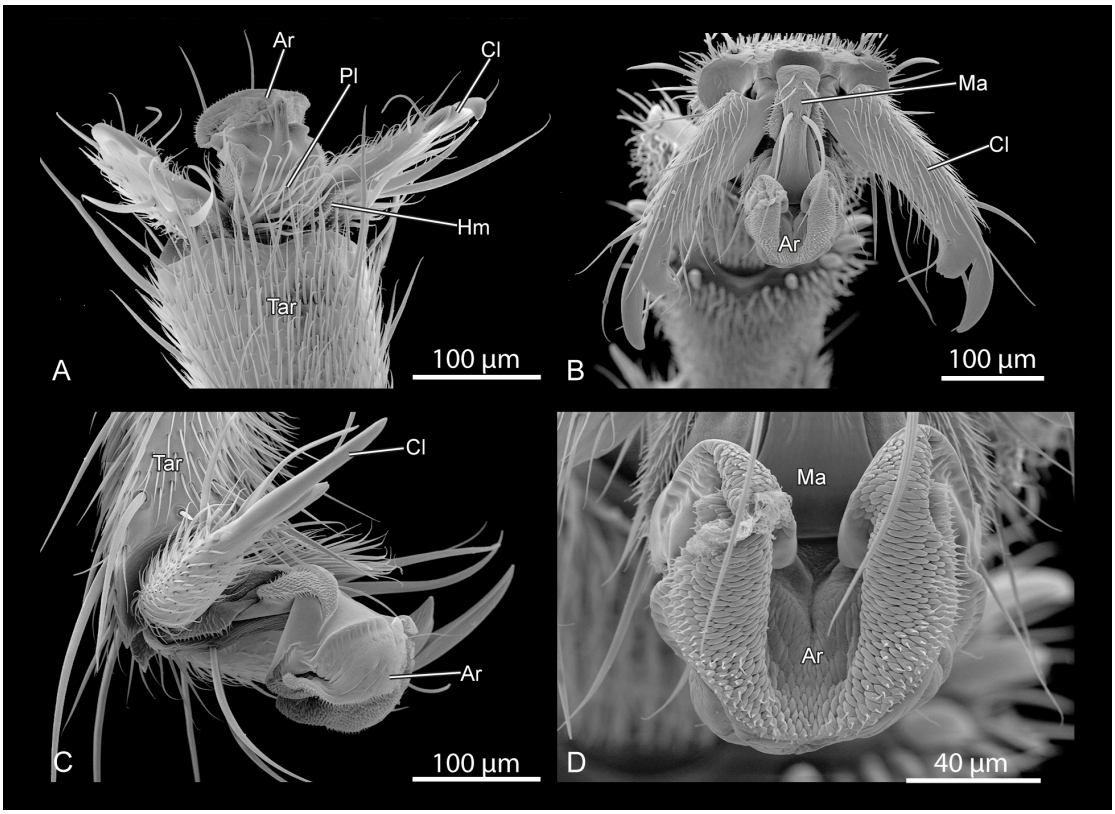

Fig. 6. *Myrmecia nigrocincta*, pretarsal structures, SEM micrographs. **A**: dorsal view; **B**: front view; **C**: lateral view; **D**: arolia.

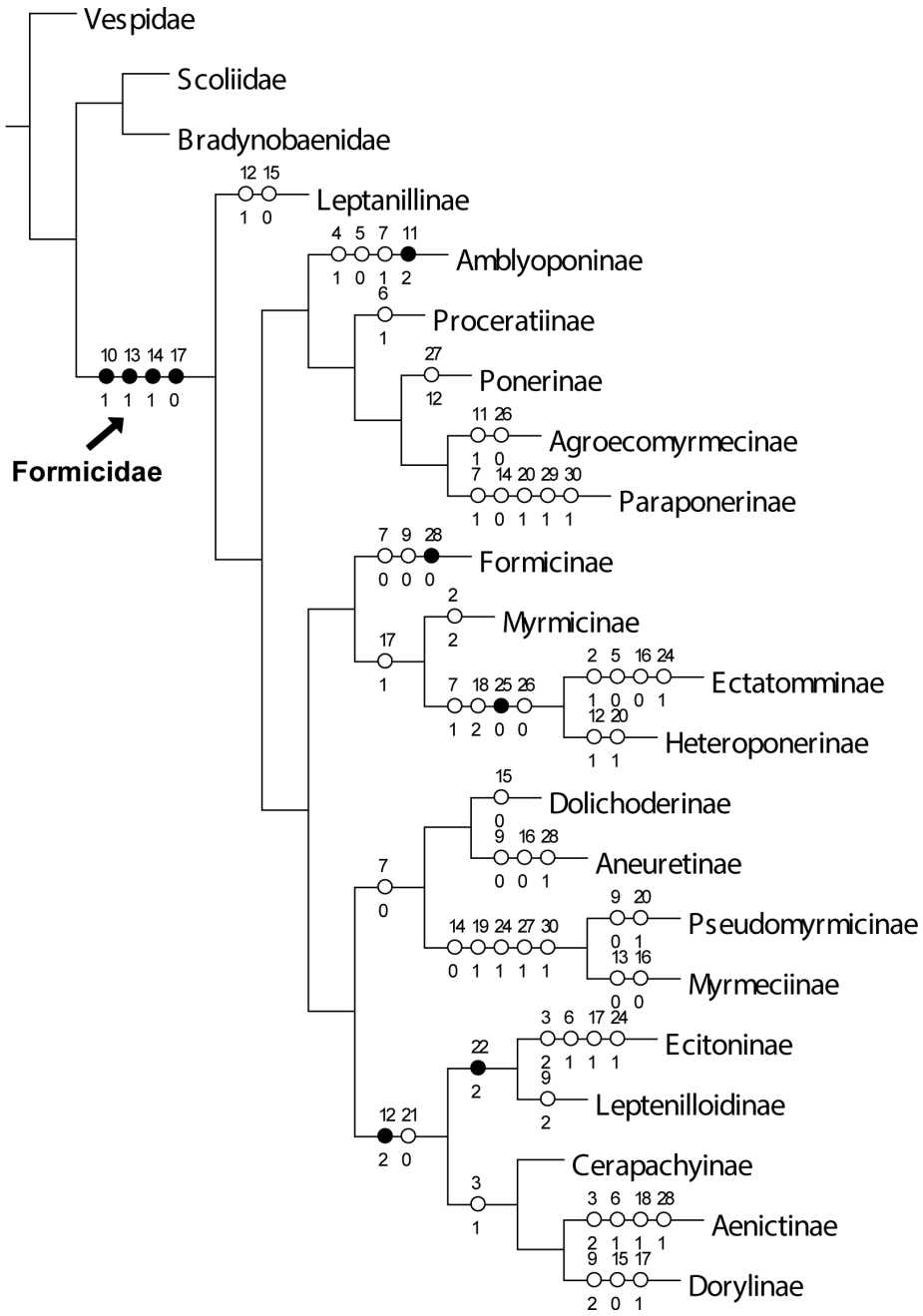

Fig. 7. Cladogram of combined analysis of 30 thoracic skeletomuscular characters based on a molecular phylogeny (MOREAU & BELL 2013). Apomorphies are mapped on the tree as circles. Character serial numbers are labeled above each circle; characters states below each circle.

3.4.2. Published Version

The mesosomal anatomy of *Myrmecia nigrocincta* workers and evolutionary transformations in Formicidae (Hymenoptera)

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Accepted 07.xii.2018.
Published online at www.arthropod-systematics.de on 00.xx.20??.
doi

Editors in charge: Andy Sombke & Klaus-Dieter Klass

Abstract. The mesosomal skeletomuscular system of workers of *Myrmecia nigrocincta* was examined. A broad spectrum of methods was used, including micro-computed tomography combined with computer-based 3D reconstruction. An optimized combination of advanced techniques not only accelerates the acquisition of high quality anatomical data, but also facilitates a very detailed documentation and visualization. This includes fine surface details, complex configurations of sclerites and also internal soft parts, for instance muscles with their precise insertion sites. Myrmeciinae have arguably retained a number of plesiomorphic mesosomal features, even though recent molecular phylogenies do not place them close to the root of ants. Our mapping analyses based on previous morphological studies and recent phylogenies revealed few mesosomal apomorphies linking formicid subgroups. Only five apomorphies were retrieved for the family, and interestingly three of them are missing in Myrmeciinae. Nevertheless, it is apparent that profound mesosomal transformations took place in the early evolution of ants, especially in the flightless workers. The modified mesosoma is characterized by four character complexes: **a)** an enlarged prothorax with elongate procoxae, a large plate-like pronotum, strongly developed muscles of the forelegs and especially of the neck region; **b)** highly differentiated legs with complex cleaning and attachment devices; **c)** a reduced flight apparatus with greatly simplified pterothoracic musculature and mechanically reinforced exoskeleton and **d)** strongly developed specialized muscles inserted on the base of the metasoma. Structural modifications of the prothorax and neck region allow ant workers to transport items efficiently with a highly movable head with strongly developed cervical muscles. Their differentiated legs enable them to move efficiently on various surfaces and to maintain their complex apparatus of sensilla. The mechanically reinforced mesosoma provides protection against predators and likely against detrimental environmental agents. The enhanced movability of the metasoma increases the defensive capacity with a sting or other mechanisms.

Keywords. *Myrmecia*, Formicidae, ants, mesosoma, phylogeny, evolution.

1. Introduction

Even though most individuals of Formicidae are small and inconspicuous, the group is exceptionally popular and also generally known outside the community of entomologists. The diversity of the family is relatively modest with ca. 12 800 described species (BOUDINOT 2015). However, the enormous biomass, the obviously high impact in many ecosystems, elaborate forms of eusociality and complex behavior patterns have attracted intensive attention of researchers since the early days of scientific entomology (see e.g. HÖLLDOBLER & WILSON 1990). Morphological research on Formicidae goes back to the late 19th century (e.g. NASSONOFF 1889). Nevertheless, considering the enormous popularity and importance of the group, the available anatomical data are surprisingly scarce. Most morphological investigations were restricted to external skeletal features, whereas detailed and well-documented anatomical studies are still scattered and limited in their taxonomic scope.

A very early study providing anatomical data on "ants, bees and wasps" was presented by LUBBOCK (1881). The morphology of external mesosomal structures of ants was discussed by NASSONOFF (1889) and JANET (1898). EMERY (1900) homologized structures in different ant castes, and his nomenclature was used by later researchers. SNODGRASS (1910a) briefly described the mesosoma of an ant worker in a comprehensive study on Hymenoptera. TULLOCH (1935) compared external mesosomal structures of alates and workers. The anatomy and life history of workers of *Camponotus herculeanus pennsylvanicus* De Geer, 1773 were described by FORBES (1938) and the anatomy of *Rhytidopenera metallica* (Smith, 1858) by WHELDEN (1960). Workers from seven subfamilies were described by REID (1941) for a general comparison of wingless and short-winged types in Hymenoptera. MARKL (1966) and SAINI et al. (1982) described the mesosomal skeletomuscular system of single species, the former also covering the mesosomal nervous system. The tracheal system was investigated by KEISTER (1962). DE GUSMÃO et al. (2001), BILLEN et al. (2011) and BILLEN (2017) examined the metapleural gland under morphological and functional aspects.

Many studies on external structures were used in a taxonomic context (e.g. WILSON et al. 1967; GOTWALD & KUPIEC 1975; GOTWALD & SCHAEFER 1982; BOLTON 2003; BOUDINOT 2015), partly also covering fossil taxa (e.g. GRIMALDI et al. 1997; ENGEL & GRIMALDI 2005; BARDEN & GRIMALDI 2012). EMERY (1877) proposed the first evolutionary hypothesis of relationships among ant subfamilies. There was a gap until BROWN (1954) presented a revised comprehensive morphology-based phylogeny, based on results of previous studies. He did not explicitly use a Hennigian (or cladistic) approach in this contribution. Nevertheless, due to his profound knowledge of the group and his extensive taxonomic work based on a series of previous investigations (e.g. EMERY 1877; WHEELER 1928), his tentative evolutionary tree is highly consistent with results of some recent analyses of molecular data (e.g. BRADY et al. 2006; MOREAU et al. 2006; KELLER 2011; WARD 2014). Using scanning electronic microscopy, BARONI URBANI et al. (1992) and KELLER (2011) characterized more detail of external morphology for explicit phylogenetic study of Formicidae. Combining anatomical data, phylogenetic

systematics and locomotor function, KELLER et al. (2014) analyzed the mesosomal evolution in ant castes and trade-off between different behavioral patterns.
Recently, computer-based 3D reconstruction was used to increase the efficiency and accuracy of the documentation of external and internal characters, and also to facilitate sharing complex morphological data (FRIEDRICH et al. 2013; WIPFLER et al. 2016). HITA GARCIA et al. (2017) applied these methods in descriptions of two new species from Madagascar. However, fine details like muscle insertions were not documented in that study, and generally the use of this approach (and other innovative methods) is still limited in studies on Formicidae and related groups. Consequently, our primary aim was to provide detailed documentation of the mesosoma of an ant worker using a broad spectrum of techniques, including microphotography, scanning electron microscopy (SEM), micro-computed tomography (μCT) and 3D reconstruction. For our investigation, we chose a species from the subfamily Myrmeciinae. Even though this group is likely not close to the root of the family (MOREAU & BELL 2013; WARD 2014; BLANCHARD & MOREAU 2017; BOROWIEC et al. 2017; BRANSTETTER et al. 2017a), it shows a high number of discernable mesosomal sclerites, and is therefore possibly close to the plesiomorphic mesosomal status of Formicidae (WARD & BRADY 2003). We homologize the mesosomal muscles observed in *Myrmecia nigrocincta* with those previously described for species of other groups, notably the honeybee (SNODGRASS 1942), but also other representative of Aculeata and taxa close to the root of the hymenopteran tree such as Xyelidae and Tenthredinidae (VILHELMSEN 2000a, b; MAKI 1938). We compare our observations with conditions found in formicid alates and members of other groups of Aculeata. Finally, we present some interpretations on the functional and evolutionary background of modifications occurring in different castes of ants.

2. Materials and methods

2.1. Specimens examined

Myrmecia nigrocincta Smith, 1858, worker, preserved in 70% ethanol, collected by R. Jordan in Australia, Queensland, Mount Hypipamee on September 2, 2002 in an open forest habitat. The species identification follows the key from OGATA & TAYLOR (1991).

2.2. Hand drawings

One specimen was manually dissected in 70% ethanol under a Zeiss Stemi SV11 with an additional Euromex Illuminator EK-1 lighting system. The mesosomal sclerites were drawn with full lines, margins below other sclerites with dotted lines. The legs were omitted, except the coxal elements. The figures were drawn with pencil under the microscope, scanned into the computer and finished with Adobe Illustrator CC.

2.3. Computer-based 3D reconstruction

One specimen was dehydrated in an ethanol series (from 70% to 100%) transferred into Acetone and dried at the critical point (EmiTech K850 Critical Point Dryer). It was scanned in a SkyScan221 micro-CT (FSU Jena) with beam setting of 40 kV and 320 µA. In a 360° scan pictures were taken every 0.2° with an exposure time of 150 ms. A pixel size of 0.9 µm was achieved. The mesosomal segments of the specimen were reconstructed three-dimensionally based on the µCT-image stack using FEI Amira 6.0 for segmentation and VG-Studio Max 2.0 (Volume Graphics GmbH, Heidelberg, Germany) for volume-/ surface renders.

2.4. Microscopic photography and scanning electronic microscopy

One specimen was air dried after fixing it in position over night in 100% ethanol. It was photographed with a Keyence VH-Z20R to record the general body shape and coloration. Another specimen was coated with gold (EmiTech K500 sputter coater) after the critical point drying. Micrographs were taken with Philips XL 30 ESEM (FEI) and ResAlta Scandium software.

2.5. Reconstruction of character evolution

External characters of the mesosoma for the mapping analysis were adopted from previous morphology-based phylogenetic studies including No. 10–15 from BARONI URBANI et al. (1992) and No. 49–94 from KELLER (2011). These two references provide comprehensive data on the external skeletal morphology. We excluded character No. 10–12 from BARONI URBANI et al. (1992) that are duplicated as characters No. 49, 60 and 62 in KELLER (2011). Based on parsimony inference we assigned the most plausible character state to the groundplan of each subfamily if it was represented by more than one terminal with variation in this feature (NINOMIYA & YOSHIZAWA 2009). In cases of ambiguity we scored multiple states for the terminal. We also used the same method to combine invalid subfamilies Ecitoninae, Leptanilloidinae, Cerapachyinae, Aenictinae and Dorylinae into one branch as Dorylinae. We then checked uninformative characters in WinClada with the function "Mop uninformative chars" and deleted them. Finally, 26 characters (Table S1) for 15 formicid subfamilies and 3 outgroup taxa Scoliidae, Bradynobaenidae and Vespidae were mapped on a manually reconstructed tree in WinClada with the function "Move branch mode", using the phylogenomic topology from BRANSTETTER et al. (2017a). It contains a large number of terminal taxa, extensive gene regions and relatively unambiguous alignments.
We also homologized the mesosomal muscles of 12 species of Hymenoptera with those observed in our studied species *M. nigrocincta* (Tables 1, S2). The muscular characters come from LUBBOCK (1881), MAKI (1938), DUNCAN (1939), SAINI et al. (1982), VIHELMSEN (2000a,b), MIKÓ et al. (2007), SNODGRASS (1942) and ALAM (1951). The information from the last two references was extracted from "TABLE XXII" of MATSUDA (1970). In total, these references cover seven families including Xyelidae, Tenthredinidae, Ichneumonidae, Braconidae, Scelionidae, Vespidae, Apidae and Formicidae. Among them, Vespidae is represented by three species of *Vespa*. Formicidae is represented by three species of Formicinae: two of the ant studies contain information from both alate gynes and flightless workers (LUBBOCK 1881; SAINI et al. 1982), while one contains only information from workers (MARKL 1966).

2.6. Terminology

The terminology for the mesosomal exoskeleton is based on REID (1941) and KELLER (2011), and on FRIEDRICH & BEUTEL (2008a) for internal skeletal structures and muscles.
Abbreviations: A – posterolateral margins of pronotum; Ar1/2/3 – arolium of fore-/mid-/hindleg; B – meso-metapleural suture; Btar1/2/3 – pro-/meso-/metabasitarsus; BtarS – basitarsus setae; Ca – calcar; CaBa – brush on anterior side of calcar; CaLa – lamella of calcar; CaPe – pectinate-shaped structure of calcar; Cl1/2/3 – claw of pro-/mid-/hindleg; Cx1/2/3 – pro-/meso-/metacoxa; Dc2/3 – meso-/metadiscrimen; Fe1/2/3 – pro-/meso-/metafemur; Fu1/2/3 – pro-/meso-/metafurca; Hm – hairy membrane, ISp – propodeal spiracle; IT – propodeum; Lcv – lateral cervical sclerites; Ma2 – manubrium of midleg; N1/2/3 – pro-/meso-/metanotum; Pl1/2/3 – pro-/meso-/metapleuron; Pl3G – metapleural gland; Pl3Go – metapleural gland orifice; Pl – planta; Ses – stout setae; Sp2/3 – meso-/metathoracic spiracle; SpL – spiracle lobe; StrC – strigil comb, StrN – strigil notch; S1 – prosternum; Tar1/2/3 – pro-/meso-/metatarsus; Tb1/2/3 – pro-/meso-/metatibia; Tr1/2/3 – pro-/meso-/metatrochanter; TspA2/3 – meso-/metatibia antero-dorsal apical setae; TspP2/3 – meso-/metatibia posterior apical setae. — Abbreviations for muscles are based on the terminology of FRIEDRICH & BEUTEL (2008a). Two newly introduced abbreviations are used, IA1 for the 1st elevator of the abdomen, IA2 for the 2nd elevator (LUBBOCK 1881).

3. Results

[Ab hier **Figs. 1-6** dicht einsetzen;
Fig. 1 seitenbreit, Legende drunter; tendenziell etwas vor 3. Results;
Fig. 2 ¾ seitenbreit, Legende drunter;
Fig. 3 seitenfüllend, aber Legende noch drunter [Figs. 2 und 3 bitte auf gegenüberliegenden Seiten];
Fig. 4 seitenfüllend, Legende auf Seite gegenüber;
Fig. 5 seitenfüllend, aber Legende noch drunter;
Fig. 6 seitenbreit, Legende drunter**]**

3.1. Skeletal structures

3.1.1. General appearance

The entire tagma appears very slender in dorsal view, and greatly simplified in its entire skeletal configuration compared to more generalized insects (e.g. Zoraptera; FRIEDRICH & BEUTEL 2008a). It is densely covered by pubescence (Fig. 3). In lateral view the pro- and mesothorax form an arch from the strongly narrowed cervical region to the mesocoxal insertion area. The propodeum appears parallelogram-shaped in lateral view, resting like a saddle on the posterior mesothorax. The strongly slanting and very distinct meso-metapleural suture (REID 1941) (Figs. 2C, 3C, 4A: B) extends from the metathoracic spiracle to the lateral mesocoxal articulation.

3.1.2. Cervical region and prothoracic skeleton

The segment appears elongated and pear-shaped in dorsal view, and slightly curved in lateral view, with an evenly curved, ascending pronotum and a long concave anteroventral margin. The cuticle is slightly rugose, without any conspicuous surface modifications.
The cervical membrane connects the very narrow foramen occipitale with the narrow anterior prothoracic margin. The wide and concave surface of the occipital region of the head appears very large in comparison to the anterior prothorax. The neck region lacks exposed sclerotized cervicalia, but an invaginated internal process represents a slightly bulged lateral cervical sclerite (Fig. 4D: Lcv).
The elongate pronotum (Figs. 1A,B, 2A,C, 3A,C, 4A: N1) is evenly rounded along its lateral margins; it reaches its greatest width at its posterior 1/4 and very distinctly narrows before it connects with the mesonotum, with posterolateral edges, which appear evenly rounded in dorsal view (Figs. 2C, 3C, 4A: A); the anterior margin is slightly bent upwards; it is distinctly narrowed, about 1/3 as wide as the maximum width of the segment; the lateral edge of the pronotum is rounded; the posterior edge appears more or less concave depending on the angle of view; the prophragma at the hind margin of the pronotum is distinctly reduced.
The large propleuron (Figs. 1B,C, 2B,C, 3B,C, 4A: Pl1) is inflexed medially and curving posteroventrally, thus covering the lateral part of the segment and also the entire ventral area, where both parts meet along a ventromedian line; a distinct, evenly curved articulation separates it from the pronotum, running almost exactly parallel to the lateral segmental border; antero-laterally it forms paired roughly triangular projections, enclosing a V-shaped incision, which forms the propleural antero-ventral edge of the segment.
The strongly elongated procoxae (Figs. 1B,C, 2B,C, 3B,C, 4A: Cx1) extend postero-ventrad from the posterior propleural margin; they are inserted in transversely oval procoxal cavities. Antero-medially the profurca (Fig. 4B: Fu1) arises from a rhombic prosternum (Figs. 2B, 3B: S1) between the procoxae, the only exposed sternal element; a pair of extensive processes for muscle attachment arise from the anterior side of the apical part of the profurca.

3.1.3. Mesothoracic skeleton

The mesonotum (Figs. 1A,C, 2A,C, 3A,C, 4A: N2) is a completely undivided, moderately large, oval sclerite; it is about 1.5 x as long as wide and evenly rounded on all sides; antero-laterally it bears a pair of mesothoracic spiracles (Figs. 2C, 3C, 4A: Sp2), which are covered by semicircular spiracle lobes (Figs. 2C, 3C, 4A: SpL). The large mesopleuron (= mesoplectus) (Figs. 1B,C, 2B,C, 3B,C, 4A: Pl2) is dorsally continuous with the lateral mesonotal margin; it expands over the entire lateral and ventral areas; ventro-medially it is invaginated into the mesosomal lumen to form an elongate-triangular mesodiscriminal lamella (Fig. 4B: Dc2), which is posteriorly connected to the mesofurcal base. The nearly spherical mesocoxae are distinctly shorter than the procoxae and laterally directed in their resting position (Figs. 1B,C, 2B,C, 3A–C, 4A: Cx2); they are inserted at the posterior margin of the mesothoracic venter; they are almost adjacent medially, even though the internal openings of the mesocoxal cavities are widely separated

and distinctly smaller than their prothoracic counterparts. The narrow mesofurca (Fig. 4B: Fu2) is curved in lateral view; it originates from the middle area between the paired mesocoxae; from there it extends antero-dorsad and connects to the dorsal part of the mesopleuron; a slender antero-median mesofurcal process serves as attachment area of a ventral longitudinal muscle (Fig. 4C: Ivlm7).

3.1.4. Skeleton of metathorax and propodeum

The metanotum (Figs. 2A, 3A: N3) is a very narrow and undivided element between the annular metathoracic spiracles (Figs. 2A,C, 3A,C, 4A: Sp3), which are located close to the postero-lateral mesonotal margin. The large propodeum (Figs. 1A, C, 2A,C, 3A,C, 4A: IT) anteriorly connects with the metanotum, and ventrally with the large triangular metapleura (Figs. 2A, C, 3B, 4A: Pl3). The paired metapleura expand over the entire lateral and ventral areas, and a sclerotized metadiscrimen (Fig. 4C: Dc3) extends from the ventral midline between the paired metapleural halves upward into the mesosomal lumen. Dorso-laterally, paired slit-shaped propodeal spiracles are present (Figs. 2A,C, 3A,C, 4A: ISp), and the paired orifices (Figs. 2C, 3C, 4A: Pl3Go) of the flocculent metapleural glands (Fig. 4E: Pl3G) are located postero-laterally. Externally, a low carina is present antero-dorsally to the gland opening. The conical metacoxae (Figs. 1B,C, 2B,C, 3A–C, 4A: Cx3) are longer than the mesocoxae; they are inserted at the posterior edge of the metathoracic venter; the internal openings of the metacoxal cavities are medially connected, thus forming a broad, transverse postero-ventral aperture. The narrow metafurca (Fig. 4B: Fu3) originates from the median area between the metacoxae and extends antero-dorsad along the anterior metadiscrimen; anteriorly they connect to the postero-dorsal part of mesofurca; this part forms a semi-cylindrical structure that ensheaths the very long and slim ventral longitudinal muscles (Fig. 4C: IIvlm7).

3.1.5. Legs

All three pairs of legs are long and slender and covered by short pubescence. Additionally, fine long setae are dispersed over the entire surface, with their length decreasing distally. The foreleg is the shortest and the hindleg the longest. However, the procoxa (Figs. 1B,C, 2C, 3C, 4A, 5A: Cx1) is distinctly elongate, almost twice as long as its meso- (Figs. 1B,C, 2C, 3C, 4A, 5B: Cx2) and metathoracic counterparts (Figs. 1B,C, 2C, 3C, 4A, 5C: Cx3). All trochanters (Fig. 5A–C: Tr1–3) are short, barrel-shaped sclerites. The protrochanter connects laterodistally with the procoxa, probably allowing rotatory movements; the meso- and metatrochanters connect with the distal ends of their respective coxae with a hinge-like contact zone, likely resulting in restricted movability. The femur (Figs. 1A–C, 5A–C: Fe1–3) is the longest element of all three legs. It is slightly bulbous in its proximal half, especially in the fore- and hindlegs, and it narrows distally. The femuro-tibial articulations allow movements in one plane (Fig. 5A). The tibiae (Figs. 1A–C, 5A: Tb1) are rather straight and narrower than the femur. All tarsi (Figs. 1A–C, 5A: Tar1) are pentamerous; the basitarsus is about as long as the remaining tarsomeres combined; tarsomere 4 is the shortest and is bilobed; tarsomere 5 bears paired claws and an arolium (Fig. 5A).

A complex armature is present on the distal parts of all three legs, but most elaborate on the foreleg. The protibia bears a row of four stout setae distally on its posteroventral surface; the posterior side bears three stout setae apically near the calcar (Fig. 5E,F: Ca) of the strigil (FRANCOEUR & LOISELLE 1988: antenna cleaner), which articulates on the ventral side of the protibia. The calcar carries a lamella (Fig. 5F: CaLa) proximally and a pectinate-shaped structure (Fig. 5F: CaPe) on its distal region; on its anterior surface, it bears a brush of clubbed microtrichia, running parallel to the lamellate and pectinate edge and covering most of its surface on this side (Fig. 5E: CaBa); densely packed, short, spine-like microtrichia are present on the ventral surface of the calcar. The probasitarsus (Fig. 5E,F: Btar1) forms the corresponding part of the strigil; its anterior surface bears paddle-shaped setae similar to the clubbed microtrichia of the calcar; very similar setae are also inserted distally on the antero-ventral protibial surface. The probasitarsus bears shorter, apically rounded setae on its posterior and ventral side; the comb of the strigil (Fig. 5F: StrC) is inserted in a proximal notch of the probasitarsus (Fig. 5F: StrN), and a row of stout setae (Fig. 5F: Ses) extends parallel to it on the posterior surface of this tarsomere; more widely spaced stout setae are continuous with this row and reach the distal end of the probasitarsus.

The meso- and metatibia also bear stout apical setae, antero-dorsally and posteriorly on the midleg and posteriorly on the hindleg; additionally, both carry two spurs distally on the ventral side (Fig. 5G,H). The mesotibial spurs (Fig. 5G: TspA2, TspP2) are very similar to each other in length and configuration, with short, spine-like microtrichia on the ventral side and a row of cuticular teeth on the dorsal edge. The posterior metatibial spur (Fig. 5H: TspP3) is longer and stronger than the anterior one (Fig. 5H: TspA3), and is somewhat similar to the strigil; in contrast, the smaller anterior spur is similar to its mesotibial counterpart, the larger posterior spur carries a comb of microtrichia on its dorsal side, quite similar to that of the calcar; additionally, this spur bears a small brush of club-like microtrichia on its posterior side, smaller than the one on the anterior side of the calcar but otherwise similar; paddle-shaped setae are inserted on the postero-ventral metabasitarsus, similar to those of the anterior probasitarsal surface. A group of blunt setae is inserted distally on the postero-ventral metatibial surface. The meso- and metabasitarsi bear lateral rows of stout setae like the probasitarsus, additionally a sulcus is present on their anterodorsal surface (Fig. 5D: BtarS).

The terminal parts of all legs are similar and well-developed (Fig. 6). They articulate with the distal rim of tarsomere five and bear an apically rounded preapical tooth that is broader than the apical part. A vestiture of short setae is present on the proximal two thirds of the claws (Fig. 6A–C: Cl1–3), three long setae are inserted ventro-laterally, and a field of minute hairs is present on the ventral base. The arolium (Fig. 6A–D: Ar1–3) and its supporting sclerites originate between the claws; the humerus-shaped manubrium (Fig. 6B,D: Ma2) is dorsally articulated with tarsomere 5; it bears several small setae proximally and two larger setae proximad its middle region; membranous areas with minute spine-like protuberances are present at the lateral base of the manubrium. The surface structure at the lateral membranous bases of the arolium is spinose, whereas its ventral and lateral regions are smooth. The deeply concave dorsal surface resembles a wicker basket, with scale-like microtrichia along most of its dorsal surface; some of these minute scale-like structures, especially close to the margin, bear a single minute microtrichium on the tip. Ventrally, the planta (Fig. 6A: Pla1)

is densely covered with setae. The unguitractor plate could not be observed with the techniques we applied and the arcus is also concealed (FEDERLE et al. 2001).

3.2. Musculature (Fig. 4)

3.2.1. Prothoracic muscles

Dorsal longitudinal muscles. Idlm1, *M. prophragma-occipitalis*, long and slender, slightly wider in its middle region; O (= origin): median part of posterior pronotal margin, I (= insertion): dorsally on posterior edge of occipital region, close to midline. — **Idlm5**, *M. pronoto-phragmalis anterior*, fan-shaped, strongly narrowing anteriorly, narrower posteriorly; O: postero-lateral area of pronotum; I: latero-median part of posterior pronotal margin.
Dorsoventral muscles. Idvm5, *M. pronoto-cervicalis anterior*, broad muscle, narrowing towards insertion site on lateral cervical sclerite; O: antero-lateral area of pronotum; I: lateral cervical sclerite. — **Idvm9**, *M. profurca-occipitalis*, long slender bundle; O: anterior area of profurca; I: dorsal occipital region. — **Idvm18**, *M. pronoto-coxalis lateralis*; O: postero-lateral area of pronotum; I: lateral procoxal rim.
Tergo-pleural muscles. Itpm1, *M. pleurocrista-occipitalis*, very large muscle, narrowing towards insertion on occipital region; O: postero-lateral area of propleuron; I: dorso-median area of occipital region. — **Itpm2**, *M. propleuro-occipitalis*, very large muscle, larger on propleuron, narrowing towards insertion on occipital region; O: postero-dorsal area of propleuron; I: dorso-median area of occipital region. — **Itpm4**, *M. pronoto-apodemalis anterior*; O: ventro-lateral area of pronotum; I: dorsal area of profurca. — **Itpm5**, *M. pronoto-apodemalis posterior*; O: postero-lateral area of pronotum; I: profurcal anterior process. — **Itpm6**, *M. pronoto-intersegmentalis*; O: postero-ventral area of pronotum; I: basal area of profurca.
Pleuro-coxal muscles. Ipcm4, *M. propleuro-coxalis superior*, triangular, narrowing towards insertion on procoxal rim; O: postero-dorsal area of proepimeron; I: lateral procoxal rim. — **Ipcm8**, *M. propleuro-trochanteralis*, long muscle; O: postero-dorsal area of proepimeron; I: protrochanteral tendon.
Ventral longitudinal muscles. Ivlm1, *M. profurca-cervicalis*, slender muscle; O: profurcal anterior process; I: lateral cervical sclerite. — **Ivlm3**, *M. profurca-tentorialis*, long slender bundle; O: anterior area of profurca; I: ventral occipital region. — **Ivlm7**, *M. profurca-mesofurcalis*, two subcomponents, the median bundle slender and shorter, the lateral one widening towards mesofurcal insertion site; O: posterior area of profurca; I: median bundle on anterior mesofurcal process, lateral subunit on antero-dorsal part of mesofurca.
Sterno-coxal muscles. Iscm1, *M. profurca-coxalis anterior*; O: postero-ventral area of propleuron close to the basal part of profurca; I: anterior procoxal rim. — **Iscm3**, *M. profurca-coxalis medialis*, wider on profurca, narrowing towards insertion on procoxal rim; O: dorsal part of profurca; I: mesally on procoxal rim. — **Iscm4**, *M. profurca-coxalis lateralis*, triangular muscle, wider on profurca, narrowing towards insertion on procoxal rim; O: dorsal part of profurca; I: laterally on procoxal rim. — **Iscm6**, *M. profurca-trochanteralis*, long muscle; O: dorsal part of profurca; I: protrochanteral tendon.

3.2.2. Mesothoracic muscles

Dorsoventral muscle. IIdvm5, *M. mesonoto-coxalis posterior*, very large muscle, narrowing towards insertion on mesocoxal rim; O: posterior margin of mesonotum; I: laterally on mesocoxal rim.
Ventral longitudinal muscle. IIvlm7, *M. mesofurca-abdominosternalis*, very long and slender; O: postero-dorsal part of mesofurca; I: antero-ventral margin of petiole.
Sterno-coxal muscles. IIscm1, *M. mesofurca-coxalis anterior*, very large triangular muscle, narrowing towards insertion on mesocoxal rim; O: ventral area of mesopleuron; I: anteriorly on mesocoxal rim. — **IIscm3**, *M. mesofurca-coxalis medialis*, triangular muscle, narrowing towards mesocoxal rim; O: basal part of mesofurca; I: mesocoxal rim. — **IIscm4**, *M. mesofurca-coxalis lateralis*, very large triangular muscle, narrowing towards on mesocoxal rim; O: ventral area of mesopleuron; I: laterally on mesocoxal rim.

3.2.3. Muscles of metathorax and propodeum

Dorsoventral muscles. IIIdvm4, *M. metanoto-coxalis anterior*, large muscle, narrowing towards metacoxal rim; O: posterior margin of metanotum; I: antero-laterally on metacoxal rim. — **IIIdvm5**, *M. metanoto-coxalis posterior*, very large muscle, narrowing towards metacoxal rim; O: antero-lateral area of propodeum; I: postero-lateral metacoxal rim.
Ventral longitudinal muscle. IIIvlm2, *M. metafurca-abdominosternalis*, slender muscle; O: postero-ventral part of metafurca; I: antero-lateral margin of petiole.
Sterno-coxal muscles. IIIscm1, *M. metafurca-coxalis anterior*, large triangular muscle, narrowing towards metacoxal rim; O: ventral area of metafurca; I: laterally on metacoxal rim. — **IIIscm4**, *M. metafurca-coxalis posterior*; O: postero-ventral area of metafurca; I: laterally on metacoxal rim.
Muscles of propodeum. IA1, *1st elevator of abdomen*, long feather-shaped muscle; O: dorso-medial area of propodeum; I: antero-dorsal margin of abdominal segment II. — **IA2**, *2nd elevator of abdomen*; O: dorso-lateral area of propodeum; I: antero-ventral margin of abdominal segment II.

4. Discussion

4.1. Morphological techniques

The anatomical investigations in this study were carried out with only five specimens. Nevertheless, the workflow and combination of different techniques allowed a very detailed documentation of skeletal structures and also internal soft parts. This demonstrates that a combination of traditional and modern techniques can greatly facilitate the acquisition of detailed morphological data. Traditional anatomical studies can be of great value and some of them are highly accurate and detailed (e.g. DUNCAN 1939; MARKL 1966; SAINI et al. 1982; MIKÓ et al. 2007). However, information on coloration and 3-dimensional effects are usually limited or lacking (e.g. MAKI 1938). Complex anatomical illustration usually requires great experience and outstanding drawing skills (e.g. LUBBOCK 1881; WEBER 1969; see

ALLGAIER 2008). Besides this, especially in the case of small or very small objects, structural details may be easily overlooked without high quality microtome sections or µ-CT data. In the case of the ant thorax, this may concern minute muscles of the wing base, which are generally difficult to observe based only on dissections.

Microphotography accurately records important characters including coloration (also a tentative indicator of sclerotization) and transparency of cuticle (WIPFLER et al. 2016). Accompanying the written description, microphotographs provide a more intuitive impression of habitus and configuration of an insect specimen (Fig. 1). They are very well suited for gradual changes of color and texture of the cuticle, which are very difficult to reproduce with traditional drawing techniques.

Scanning electron microscopy (SEM) provides precise information on fine surface structures (FRIEDRICH et al. 2014; WIPFLER et al. 2016). Compared with hand drawn figures (Fig. 2), SEM micrographs very clearly depict minute microtrichia or scales with stereo effect as well as fine wrinkles or other patterns of microsculpture (Fig. 3). High magnification and resolution allows the documentation of fine details not accessible with other methods (Figs. 5E–H; 6: e.g. microtrichia and tiny hairs on the legs and claws), allowing their interpretation in a functional and evolutionary context. Handling of specimens and working with limited material is facilitated by a recently developed rotatable specimen holder (POHL 2010), additional advantages of this device are reduced charging of surfaces and a homogenous black background.

Microcomputed tomography (µ-CT) can greatly accelerate the acquisition of anatomical data and is less cost intensive than histological sectioning (WIPFLER et al. 2016). High quality µ-CT data are an ideal basis for 3D reconstruction, which has arguably triggered a renaissance of insect anatomy in the last 15 years (FRIEDRICH & BEUTEL 2008b; FRIEDRICH et al. 2014; WIPFLER et al. 2016). With µ-CT and suitable reconstruction (e.g. Amira, VGStudio) in an optimized workflow, external and internal structures such as sclerites, muscles, nerves and gland can be very clearly visualized (Fig. 4; WIPFLER et al. 2016). The description of the new species *Teratataner balrog* Hita Garcia, 2017 from Madagascar was based on this method (HITA GARCIA et al. 2017: fig. 11). The entire study is an exemplary contribution in taxonomy, even though fine details like muscle insertions or nerves were not identified separately.

A systematic morphological database was established by YODER et al. (2010: Hymenoptera Anatomy Ontology) for the megadiverse Hymenoptera (WIPFLER et al. 2016). Websites such as **antweb. org** provide numerous high quality images of Formicidae with standardized terminology. The enormous potential of these anatomical data collections in a taxonomic and phylogenetic context was emphasized by WIPFLER et al. (2016), and has been apparent in the works of ant systematists (e.g., YOSHIMURA & FISHER 2007; HITA GARCIA & FISHER 2011 [taxonomy]; BOUDINOT 2013 [morphology]). The technical procedures and the workflow presented in this study may contribute to the optimization of these ventures in the future.

4.2. Phylogenetic patterns and the “ancestral status” of Myrmeciinae

The first phylogenetic study of Formicidae was presented by EMERY (1877), who considered Dorylinae as most similar to the ancestral pattern “groundplan” of ants.

This hypothesis remained unchallenged until BROWN (1954) suggested a concept with this subfamily as a possible derivative from a "poneroid" ancestor, and a phylogenetic affinity with Myrmeciinae, with this taxon nested within a unit also including Pseudomyrmecinae, dolichoderomorphs and Formicinae. This phylogenetic topology remained a more-or-less preferred hypothesis (WILSON et al. 1967a,b; WILSON 1971), with some aberration (HÖLLDOBLER & WILSON 1990), until BARONI URBANI et al. (1992) conducted a formal parsimony analysis. In a study on the rediscovered Australian genus *Nothomyrmecia*, TAYLOR (1978) characterized Myrmeciinae as "the most structurally generalized ants" and their behavior as "significantly primitive" (WARD & BRADY 2003). In fact, the subfamily has arguably retained an entire series of plesiomorphic features. This includes six maxillary and four labial palpomeres in all castes, 12 antennal annuli in females and 13 in males, paired spurs on the middle and hind tibiae, tarsal claws with a strongly developed median tooth, a furcula and two-segmented gonostyli associated with the stinging apparatus, and an active closing mechanism of the proventriculus of workers (TAYLOR 1978). In an unpublished phylogenetic hypothesis from BROWN's laboratory (around 1986), BROWN intuited that Myrmeciinae were placed close to the root of the phylogenetic tree of ants, conform with an apparent proximity to the groundplan of Formicidae (KELLER 2011: fig. 4). Subsequently, taxonomic concepts have changed with the application of phylogenetic models to molecular data (e.g. BRADY et al. 2006; RABELING et al. 2008; KÜCK et al. 2011; WARD 2014; BOROWIEC et al. 2017; BRANSTETTER et al. 2017b). Moreover, new ant fossils were discovered, likely belonging to the stem-group of the family, potentially shedding new light on the ancestral morphology of extant Formicidae (e.g. GRIMALDI et al. 1997; ENGEL & GRIMALDI 2005; PERRICHOT et al. 2008; BARDEN & GRIMALDI 2012, 2016; LAPOLLA et al. 2013; BARDEN 2017). These paleontological studies provide new morphological data, which are relevant in the context of ancestral features and the early evolution of the group. Therefore, the "ancestral status" of Myrmeciinae and the groundplan of Formicidae deserve further discussion.

The monophyly of Formicidae is consistently supported in analyses based on morphological (BROTHERS 1975) or molecular data (MOREAU et al. 2006; BRADY et al. 2006; RABELING et al. 2008; MOREAU & BELL 2013; BOROWIEC et al. 2017; BRANSTETTER et al. 2017a,b). In contrast, the branching pattern within the group is affected by changing phylogenetic approaches, with different phylogenies suggested by morphological characters and molecular data sets. BARONI URBANI (1989) and BARONI URBANI et al. (1992) placed Formicinae as an early branch in studies based on morphology (BARONI URBANI 1989: fig. 2, 1992: fig. 5), suggesting that some generalized formicid features were conserved in this subfamily. The analyses of extensive to extremely large molecular data sets with broad taxon sampling (RABELING et al. 2008; KÜCK et al. 2011; MOREAU & BELL 2013; BOROWIEC et al. 2017; BRANSTETTER et al. 2017a) have placed the small and specialized subfamilies Martialinae and Leptanillinae as the first two branches in a tree of extant ant taxa, followed by formicoids with 9 subfamilies (e.g. Formicinae, Myrmicinae), and the poneroids in a large clade with 6 other subfamilies, among them Amblyoponinae and Proceratiinae. In re-analyses of morphological data with fossil taxa included, a basal position of the extinct Sphecomyrmine in Formicidae s.l. (including stemgroup taxa) was supported (GRIMALDI et al. 1997). Preserved plesiomorphies of this group are the presence of two mandibular teeth and an

antenna with a short scape and long, flexible funiculus (DLUSSKY 1983; GRIMALDI et al. 1997). Recently, another parsimony analysis placed the Cretaceous counterpart of modern trap-jawed ants haidomyrmecines as the basal branch (BARDEN & GRIMALDI 2016).

Recent phylogenetic results (BRADY et al. 2006; MOREAU et al. 2006; WARD 2014; BOROWIEC et al. 2017; BRANSTETTER et al. 2017a) clearly show that Myrmeciinae are not a "basal" group of ants, but in fact separated by six to seven nodes from the root of the tree. However, this does not exclude that the subfamily has preserved more ancestral features than most other formicid subgroups, as pointed out by TAYLOR (1978). In contrast to Myrmeciinae, the "basally" placed Martialinae are highly specialized, with an array of apomorphies linked with hypogaeic, and predacious habits, aside from some retained plesiomorphic ant features (RABELING et al. 2008; BOUDINOT 2015).

[Fig. 7 in Verbindung mit dem Folgenden, ¾ seitenbreit, Legende in Ecke links unten**]**

Our mapping analyses (Fig. 7) using the phylogeny of BRANSTETTER et al. (2017a) yields only five apomorphies of Formicidae. The exposed metathoracic spiracle with a round or oval shape [6.1] might be a derived groundplan feature, while concealment of the spiracle by a spiracular lobe has apparently evolved as an apomorphy of "core formicoids". The presence of metapleural glands [8.1] is very likely an autapomorphy of Formicidae (WHEELER 1928; WILSON et al. 1967a; BARONI URBANI 1989; BOLTON, 2003; BOUDINOT 2015). It protects adults from fungi and bacterial infections and possibly even more immatures stages (i.e., eggs, larvae, pupae) (BARONI URBANI 1989; BILLEN et al. 2011; YEK & MUELLER 2011; BILLEN 2017). Its absence in males of many species is apparently a secondary feature and probably due to their limited role in cooperative activities (HÖLLDOBLER & ENGEL-SIEGEL 1984; BARONI URBANI 1989). The propodeal spiracle without bulla [11.1], a round to oval atrial opening [12.1] and the absence of stout setae on the posterior protibial apex [15.0] were also retrieved as autapomorphies. The absence of all three features in Myrmeciinae is inferred as a result of reversals in our mapping analysis. A clade Myrmeciinae + Pseudomyrmecinae, the myrmeciomorphs of BOLTON (2003), is supported by three possible mesosomal synapomorphies, a calcar of the strigil with a basal lamella [17.1], propretarsal claws with a preapical tooth [21.1] and the presence of a metabasitarsal sulcus [26.1]. However, apparent homoplasy in these characters makes the polarity assessment ambiguous, except in the case of the last one (BOLTON 2003). This also applies to the posteriorly open metacoxal cavity [14.0], which was retrieved as an autapomorphy of Myrmeciinae. However, this trait optimized as reversal in our analysis, is very likely a retained plesiomorphy in Myrmeciinae (BOLTON 2003), with parallel evolution of losses in several formicid subgroups. Similar selective pressure may have resulted in a pattern which is less parsimonious than secondary loss in Myrmeciinae.

In addition to the derived features presented here, BOUDINOT (2015) also suggested a series apomorphies of Formicidae: disticoxal foramen directed laterally and completely enclosing protrochanteral base (char. 6); all meso- and metacoxal cavities small, circular, monocondylic, ventrally-directed disticoxae strongly produced laterally (all adult castes) (char. 7); propodeal spiracle located on lateral propodeal face distant from anterodorsal propodeal corner, often near midlength of propodeum (all adult castes) (char. 9).

4.3. Mesosomal groundplan of Formicidae

The differentiation into flightless workers and sexual morphs with preserved flight apparatus is a crucial groundplan apomorphy and key innovation of Formicidae (HÖLLDOBLER & WILSON 1990).
Compared with more generalized members of Aculeata (SNODGRASS 1910b, 1925: Apidae; DUNCAN 1939: Vespidae), alate sexual morphs of ants show only limited modifications of the mesosoma, such as for instance wings with a reduced venation, or obsolete mesopleural ridges. The pronotum is larger than the short and clasp-shaped structure of Vespidae and Apidae (SNODGRASS 1910b, 1925; DUNCAN 1939), but distinctly shorter than in workers. The propleuron, a triangular plate-like structure, articulates with its lower posterior angle with the coxa, and with the occipital region on its anterior margin. Cervicalia are fused with the propleura as in the Hymenoptera groundplan postulated by VILHELMSEN (2000a). The small but separate sclerites of the neck region identified in the honeybee by SNODGRASS (1925) are likely free due to reversal. As in Vespidae and Apidae, a functional wing articulation is preserved in the pterothoracic segments of alate ants, with axillary sclerites (SAINI et al. 1982), notal wing processes and mesothoracic basalar and subalar sclerites as attachment sites of direct flight muscles.
The highly modified mesosoma of workers, documented in detail for *M. nigrocincta* in the present study, is part of a complex of groundplan apomorphies. The pronotum is a solid and extensive plate-like structure. The propleura cover the entire lateral and ventral prothoracic regions and meet ventromedially, with only a small exposed prosternal sclerite between the procoxae. A strongly pronounced notopleural edge forms an articulation with the posterolateral head capsule. The cervical sclerite is completely fused with the internal skeleton of the propleuron. The procoxae are distinctly enlarged relative to the meso- and metacoxae in workers of *M. nigrocincta* and other groups, including stemgroup fossils (WILSON et al. 1967a,b). This is arguably an additional groundplan apomorphy of Formicidae in all adult castes, with parallel evolution in other aculeate groups such as Dryinidae (Chrysidoidea or Dryinoidea: BRANSTETTER et al. 2017b), which catch prey or hosts for their larvae with the forelegs (WALOFF 1974), and Pompilidae (Vespoidea) (VILHELMSEN, personal communication).
A crucial character complex is the complete loss of wings and flight related structures (Figs. 2, 3). The mesonotum (Figs. 1A,C, 2A,C, 3A,C, 4A: N2) forms a single sclerite without separate elements like prescutum, scutum or scutellum, and also without mesophragma (Fig. 4B). The mesopleuron (Figs. 1B,C, 2B,C, 3B,C, 4A: Pl2) is also undivided, and like in the prothorax its paired halves are fused ventromedially. Axillary sclerites, notal wing processes, basalare and subalare are missing in both pterothoracic segments of workers (Figs. 2A,C, 3A,C, 4A). The metanotum (Figs. 2A, 3A: N3) is very narrow and undivided. The metapleuron (Pl3: 2A, C, 3B, 4A) is strongly reduced. The elongated metafurca (Fig. 4B: Fu3) fuses anteriorly with the slender mesofurca (Fu2: Fig. 4B) and encloses the very slender ventral longitudinal muscle (Fig. 4C: IIvlm7).
Compared with members of other families of Hymenoptera (Tables 1, S2), the prothoracic musculature appears largely unmodified in alate and flightless ant castes. The dorsal longitudinal muscles Idlm1 and 5, the dorsoventral muscles Idvm5, 9 and 18, the tergo-pleural muscles Itpm1–5, the pleuro-coxal muscles

Ipcm1, 4 and 8, the ventral longitudinal muscle Ivlm1, 3 and 7 and sterno-coxal muscles Iscm1, 2, 4 and 6 are present in workers of *M. nigrocincta* and very likely also in the groundplan of Formicidae. As plesiomorphic features alate queens retain a relatively complete muscle set in the mesothorax. This includes well-developed dorsal longitudinal bundles and also dorsoventral, tergo-pleural and pleuro-coxal muscles (LUBBOCK 1881; SAINI et al. 1982). Among them, the presence of muscles IIdlm1, IIdvm1 and 6, and IItpm7 and 9 is likely ancestral for Formicidae. In contrast, workers, including those of *M. nigrocincta*, have lost most mesothoracic muscles, except those inserted on the mesocoxal rim and mesotrochanter. The comparison of queens and workers with representatives of other families of Hymenoptera suggests that the sterno-coxal muscles IIscm1–3 and 6 belong to the groundplan of Formicidae. The number of metathoracic muscles of queens is only slightly less than in other families of Hymenoptera (Tables 1, S2). In contrast, the muscle set is greatly simplified in workers.

Our results confirm that mesosomal groundplan features differ profoundly between ant castes, except for the procoxal-trochanteral articulations, and the meso- and metathoracic articulations with their respective coxae (BOUDINOT 2015), with moderate modifications in alate forms and far-reaching transformations in workers. Anatomical investigations of stemgroup ant fossils (e.g. GRIMALDI et al. 1997; ENGEL & GRIMALDI 2005; PERRICHOT et al. 2008; BARDEN & GRIMALDI 2012), particularly using micro-computed tomography, should have high priority. Combined with detailed morphological data on related groups, this will not only allow for a more reliable assessment of the groundplan, but also a reconstruction of early evolutionary transformations in the group.

[Table 1 in Verbindung mit 4.3., seitenhoch (bitte möglichst ganze Tabelle auf 1 Seite), Legende drüber oder auf Seite gegenüber; Farben erhalten!**]**

4.4. Mesosomal modifications in workers and their functional background

The mesosomal morphology of ant workers was apparently shaped by different but interrelated phenomena. This includes complete winglessness combined with a mechanically reinforced pterothorax, a very movable head with a strongly developed neck musculature, unusually differentiated legs as efficient cleaning tools and locomotory organs, and a highly movable gaster with a stinging apparatus preserved in *Myrmecia* and in the groundplan of the family.

The loss of wings in ant workers has consequences beyond the loss of the ability to fly. To reduce structures required in the context of flight opens perspectives to specialize in other directions (e.g. BURD 2000; SCHILMAN & ROCES 2005; BOHN et al. 2012). Although workers are “cheaply manufactured” with small and light bodies, loss of wing pairs and flight musculature, short life cycle, and lacking ovaries to support the colonial economy (PEETERS & ITO 2015), considering them as a wingless version of the alate queens would be an oversimplification (KELLER et al. 2014).

Compared with alate forms, the pronotum of workers is greatly enlarged, apparently in correlation with an enhanced muscular apparatus (KELLER et al. 2014). This increases the movability in the neck region (SNODGRASS 1935; HARTENSTEIN 2006; KELLER et al. 2014) and also enables the ants to lift and carry objects with their head, such as for instance prey, pieces of plants or seed (e.g. GORB & GORB 1999; MOLL et al. 2010; KELLER et al. 2014; NGUYEN et al. 2014).

An important modification of workers is the far-reaching fusion of sclerites. The dorsal pterothoracic sclerites are reduced to undivided and undifferentiated notal elements. Notal wing processes and axillary sclerites, important elements controlling flight movements (BRODSKY 1994), are dispensable in flightless morphs and therefore reduced, resulting in an increased mechanical rigidity of the segments. The pterothoracic sclerites are largely fused in ant workers (e.g. RICHARDS 1956; KELLER 2011). This leads to a mechanically very-compact mesosoma with a minimum of exposed membranous surfaces, a condition also occurring in beetles, even though achieved in a different way and in most cases with a retained functional flight apparatus (e.g. BEUTEL & HAAS 2000). The reinforced thorax enhances mechanical protection against predators on the ground and possibly also increases the barrier against harmful environmental agents as well as against the loss of water. Ant workers are the caste that forages outside the nest and are strongly exposed to these factors throughout their adult live span (HÖLLDOBLER & WILSON 1990; JEMIELITY et al. 2005). This results in selective pressure favoring the formation of rigid exoskeletal structures, made possible mainly by the reduction of the flight apparatus.

In a study on the mesothorax of workers, KELLER et al. (2014) noted the simplification of the mesonotum, the reduction of the mesophragma and the loss of the dorso-longitudinal muscle IIdlm1. This structural complex generally initiates the first wing depression in the flight stroke cycle in pterygote insects with retained flight ability (BRODSKY 1994), but is usually modified or distinctly reduced in secondarily flightless insects (WIPFLER et al. 2014; LIU et al. 2017). The absence of dorso-ventral muscles II/IIIdvm1 is another common feature related to the loss of the flight capacity (KOZLOV 1986; WIPFLER et al. 2014; LIU et al. 2017). As depressors of the notum during wing elevation (BRODSKY 1994), they are dispensable in flightless forms, and in fact completely absent in all examined ant workers (Tables 1, S2).

Some flight-related muscles are retained in worker ants, such as the dorsoventral muscles II/IIIdvm4, 5 and 7, which are even exceptionally well developed. These elements of the muscular system can fulfill more than one function, as wing levators in forms capable of flight, but also in the context of leg movements (KOZLOV 1986; BRODSKY 1994; LIU et al. 2017).

Linked with the loss of the flight capacity, locomotion on different substrates plays an essential role in ant workers. Consequently, the forelegs differ strongly in their structure and armature from the mid- and hindlegs (Figs. 1, 5), even though similarly well-developed muscles operating leg movements are present in all three thoracic segments. The enlarged, elongated procoxae likely allow more-efficient movements of the forelegs, together with a modified coxo-trochanteral articulation described in BOUDINOT (2015). The sterno-procoxal muscles IIscm1 and 4 distinctly are greatly enlarged in workers. This supports efficient movements on the ground including a broad range of specific activities (HÖLLDOBLER & WILSON 1990), such as jumping (CLARK 1951: 7.5–10 cm), digging (WALLIS 1962; SUDD 1969), trophallaxis, antenna cleaning (WALLIS 1962), prey handling (MASUKO 2009), grooming of queens in the case of leaf cutter ants and behavior related to maintaining hygiene in fungus gardens (FERNANDEZ-MARIN et al. 2003).

The differentiated arolia and claws further support efficient movement on various surfaces (FEDERLE et al. 2001), even though well-developed pretarsal attachment devices are a common feature in aculeate hymenopterans (FRANSTEICH & GORB

2004). The unusually complex armature of the distal leg parts, especially in the case of the fore- and hindlegs, also plays a role in different functional contexts, for instance efficient cleaning of the antennae. Perfect functioning of cuticular sensilla on the antennae and other body parts likely plays an essential role for ant workers.
A last essential character complex is the highly movable metasoma, with an aculeate stinging device retained in the groundplan of the family (HÖLLDOBLER & WILSON 1990). The increased movability of the gastral segments is supported by a strongly developed and specialized petiole musculature (Fig. 4C,D: IA1, IA2), with marked effects in the context of defense and prey capture (HASHIMOTO 1996). Protection against predators apparently plays an important role for the flightless workers. Aside from the sting, additional defensive adaptations of workers have evolved, including for instance gland secretions or sharp exoskeletal spines (e.g. BUSCHINGER & MASCHWITZ 1984; BLANCHARD & MOREAU 2017; SARNAT et al. 2017).
Structural modifications of ant workers can be seen as optimization for different tasks important for the colony. At the same time, the simplified structure of the worker thorax means "less investment" compared with winged eusocial insects with a complex flight apparatus. Highly efficient workers produced at "low cost" are likely one of the main factors contributing to the ecological dominance and success of ants (PEETERS & ITO 2015). In summary, the reduced flight apparatus of ant workers is part of an evolutionary trade-off: on one hand obvious advantages of flight like for instance dispersal, and on the other various options of flightlessness to optimize other functions in the context of a particularly successful life strategy (ROFF 1990; WAGNER & LIEBHERR 1992).

5. Acknowledgements

We are very grateful to Raul Jordan for the donation of valuable ant material. Prof. Dr. Lars Vilhelmsen (Natural History Museum of Denmark), Dr. István Mikó (Pennsylvania State University) and Prof. Dr. Lars Krogmann (Staatliches Museum für Naturkunde Stuttgart) provided valuable studies. This is also gratefully acknowledged. Meanwhile, great thanks are due to Prof. Dr. Lars Vilhelmsen and Brendon Boudinot (University of California, Davis) for their very valuable comments, which helped greatly to improve this study. This study reflects only the authors' views and the Research Executive Agency is not responsible for any use that may be made of the information it contains. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 642241. The first author Si-Pei Liu also appreciates the German Academic Exchange Service (DAAD) for the short-term grants for foreign doctoral candidates.

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Electronic Supplement Files

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File 1: liu&al-myrmeciathorax-asp2019-electronicsupplement-1.xls — **Table S1.** Character list and matrix.

File 2: liu&al-myrmeciathorax-asp2019-electronicsupplement-2.xls — **Table S2.** Muscular homology chart of Hymenoptera (Present with "+" or muscular name in green, absent with "-" in pink, uncertain with "?" or "/" in yellow. In Formicidae, the muscles only present in workers are labeled in dark green; muscles only occurring in alate castes labeled in dark blue.)

Table 1. Overview of the musculatures of Formicidae (SAINI et al. 1982; LUBBOCK 1881; MARKL 1966), Vespidae (DUNCAN 1939) and Apidae (SNODGRASS 1942). Present with "+" or muscle name in green, absent with "–" in orange, uncertain with "?" or "/" in yellow. In Formicidae, muscles present only in workers labeled in dark green, those only occurring in the alate castes in dark blue.

Family	**Vespidae**	**Apidae**	**Formicidae**			
Genus	*Vespula*	*Apis*	*Myrmecia*	*Camponotus*	*Lasius*	*Formica*
ldlm1	–	40 + 41	+	1	a	40 & 41
ldlm5	lis1[50] & lis2[51]	45	+	–	–	45
ldvm5	lpm1[37] & lpm2[38]	47	+	6	c	46 & 47
ldvm7	–	46	–	–	–	–
ldvm9	Ois2[33]	43	+	2	a1	43
ldvm18	llm6[48]	55	+	–	–	55
ltpm1	–	42b? & 42c?	+	–	b1	42/1
ltpm2	Ois1[32]	42a?	+	–	c1 & c2	42/2
ltpm3	lpm3[39] & lpm4[40]	48	–	–	–	48
ltpm4	lpm5[41]	49	+	10	e	49
ltpm5	lpm6[42]	50	+	–	f	50
ltpm6	–	–	+	–	g	–
lpcm1	llm7[49]	mcr	–	–	d1	mcr
lpcm3	llm5[47]	57	–	–	–	–
lpcm4	–	–	+	–	h	53
lpcm5	llm2[44]	53	–	–	–	–
lpcm8	llm3[45]	61	+	–	k	–
lvlm1	lfp[36]	51	+	–	d	51
lvlm3	Ois3[34] & Ois4[35]	44	+	3	b	44
lvlm7	lis4[53] & lis5[54]?	52	+	18 & 19	m & n	52
lscm1	llm1[43]	54	+	–	i1?	54
lscm2	llm4[46]	56	–	–	i	56
lscm3	–	–	+	–	–	–
lscm4	–	–	+	–	–	–
lscm5	lis3[52]	58	–	–	–	–
lscm6	llm3[45]?	61?	+	–	l	61
lldlm1	lldl1[56]	71	–	20	β	–
lldlm3	llis1[72]	70	–	–	–	–
lldvm1	lldv1[57]	72	–	21	θ	–
lldvm5	–	–	+	–	–	–
lldvm6	–	82	–	23	–	–
lldvm7	–	–	–	–	o	–
lldvm8	lldv2[58]	78	–	–	–	–
lltpm2	mut[69]	74	–	–	–	–
lltpm5	llpm4[62]	75	–	–	–	–
lltpm6	–	–	–	25	–	–
lltpm7	m3Ax[63]	76a	–	24a	–	–
lltpm9	llpm2[60] & llpm3[61]	76b & 76c	–	24b	–	–
lltpm11	llpm5[64]	–	–	–	–	–
llppm2	–	–	–	–	–	73
llspm1	llpm1[59]	77	–	–	–	–
llspm2	llfpl1[70] & llfpl2[71]	79	–	–	–	–
llpcm3	lllm1[65]	80	–	–	–	–
llvlm3	llis2[73]	–	–	–	–	79?
llvlm7	–	–	+	35	v	118
llscm1	lllm2[66]	81	+	28	p & s	81 & 82
llscm2	lllm4[68]	83	–	–	q	83
llscm3	lllm4[68]?	83?	+	29 & 31	r	83
llscm4	–	–	+	30	t	80
llscm6	lllm3a&b[67]	86	–	–	o	86
llldlm1	llldl[75]	96	–	–	–	–
llldvm1	–	–	–	–	θ	–

IIIdvm4	–	–	+	40	y?	103
IIIdvm5	–	–	+	–	y?	105
IIIdvm6	IIIpm5[80]	105	–	37	–	–
IIIdvm7	–	–	–	–	x	–
IIItpm5	IIIpm4[79]	97 & 98 & 99	–	–	–	–
IIItpm6	–	–	–	39	–	–
IIItpm7	IIIpm2[77]	100	–	38a	–	–
IIItpm9	IIIpm2[77]?	–	–	38b	–	–
IIItpm11	IIIpm3[78]	102	–	–	–	–
IIIspm1	IIIpm1[76]	101	–	–	–	–
IIIpcm2	-	-	-	36	-	-
IIIpcm3	IIIIm4[84]	103	-	-	-	-
IIIvlm2	IIIis2[86]?	118	+	33	u1	119
IIIscm1	IIIIm1[81]	104	+	41	z	104 & 105
IIIscm2	IIIIm2[82]	106	-	-	x1	106
IIIscm3	IIIIm2[82]?	106?	-	43	z1	106
IIIscm4	-	-	+	42	y1	103
IIIscm6	IIIIm3[83]	109	-	-	x	109
IA1	Iadl1[88]	120	+	34	u	120
IA2	Iadl2[89]	119	+	32	w	121

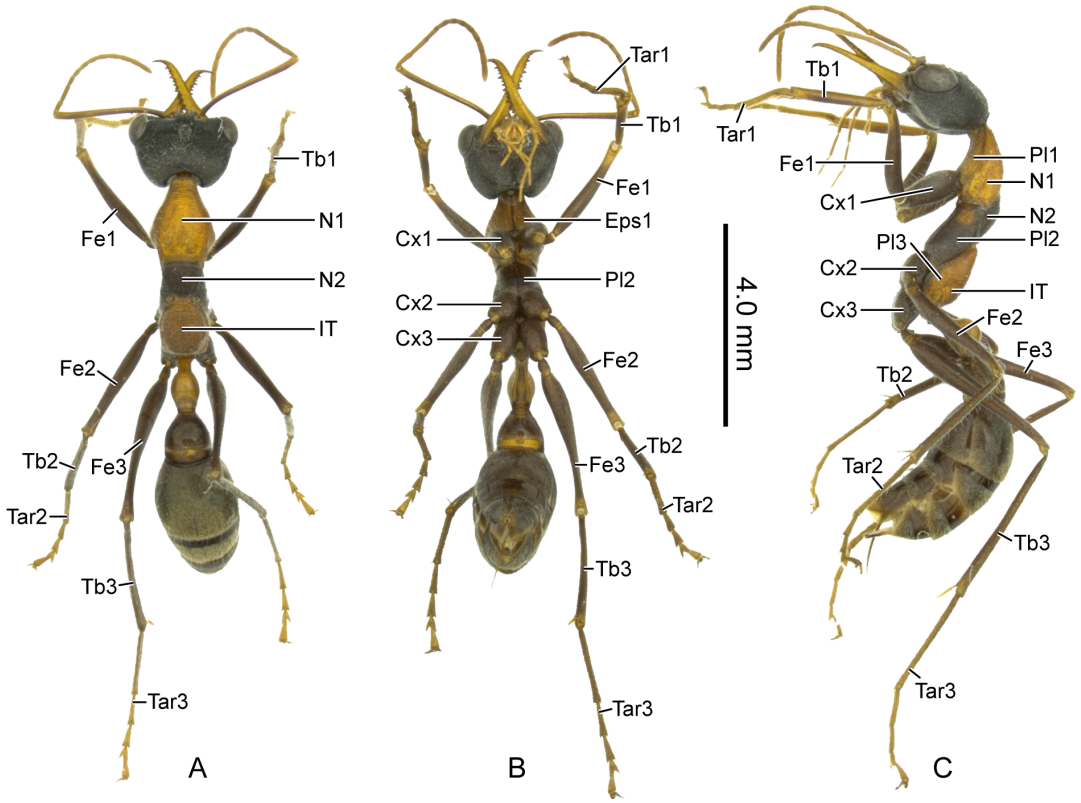

Fig. 1. *Myrmecia nigrocincta*, habitus, digital photographs. **A**: dorsal view, **B**: ventral view, **C**: lateral view.

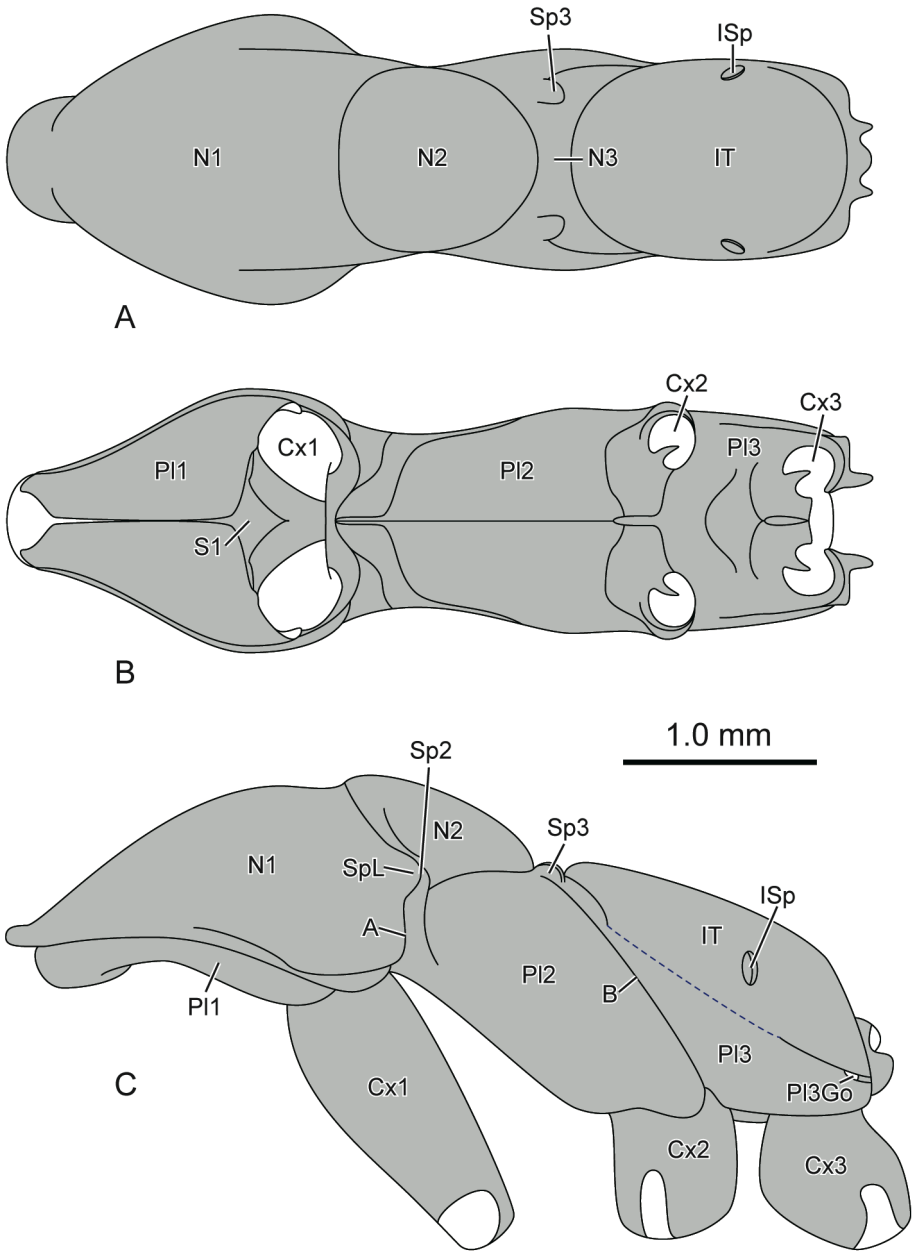

Fig. 2. *Myrmecia nigrocincta*, thoracic exoskeleton, line drawings. **A**: dorsal view, **B**: ventral view, **C**: lateral view, the dotted blue line is the border between propodeum and metapleuron.

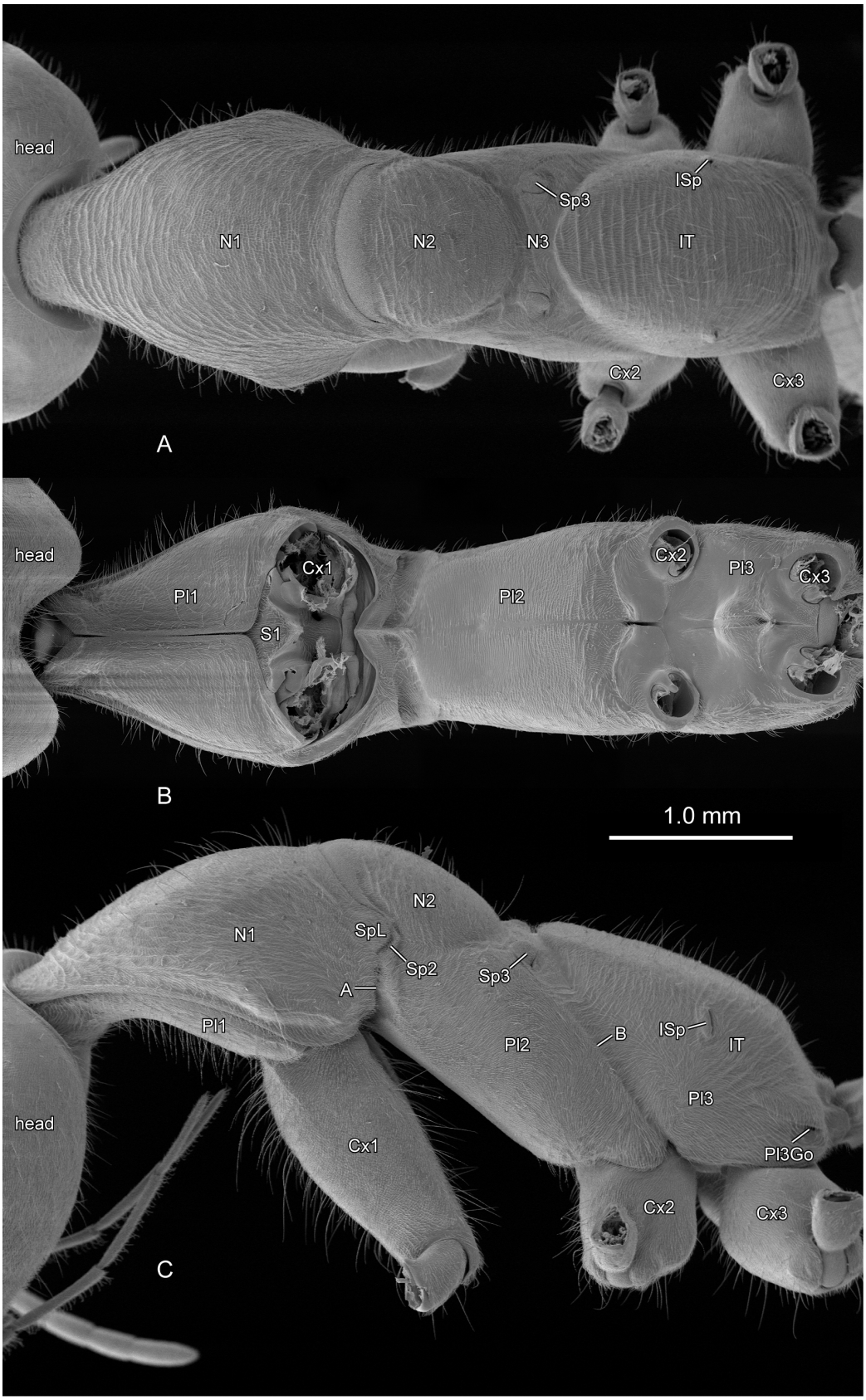

Fig. 3. *Myrmecia nigrocincta*, thoracic exoskeleton, SEM micrographs. **A**: dorsal view, **B**: ventral view, **C**: lateral view.

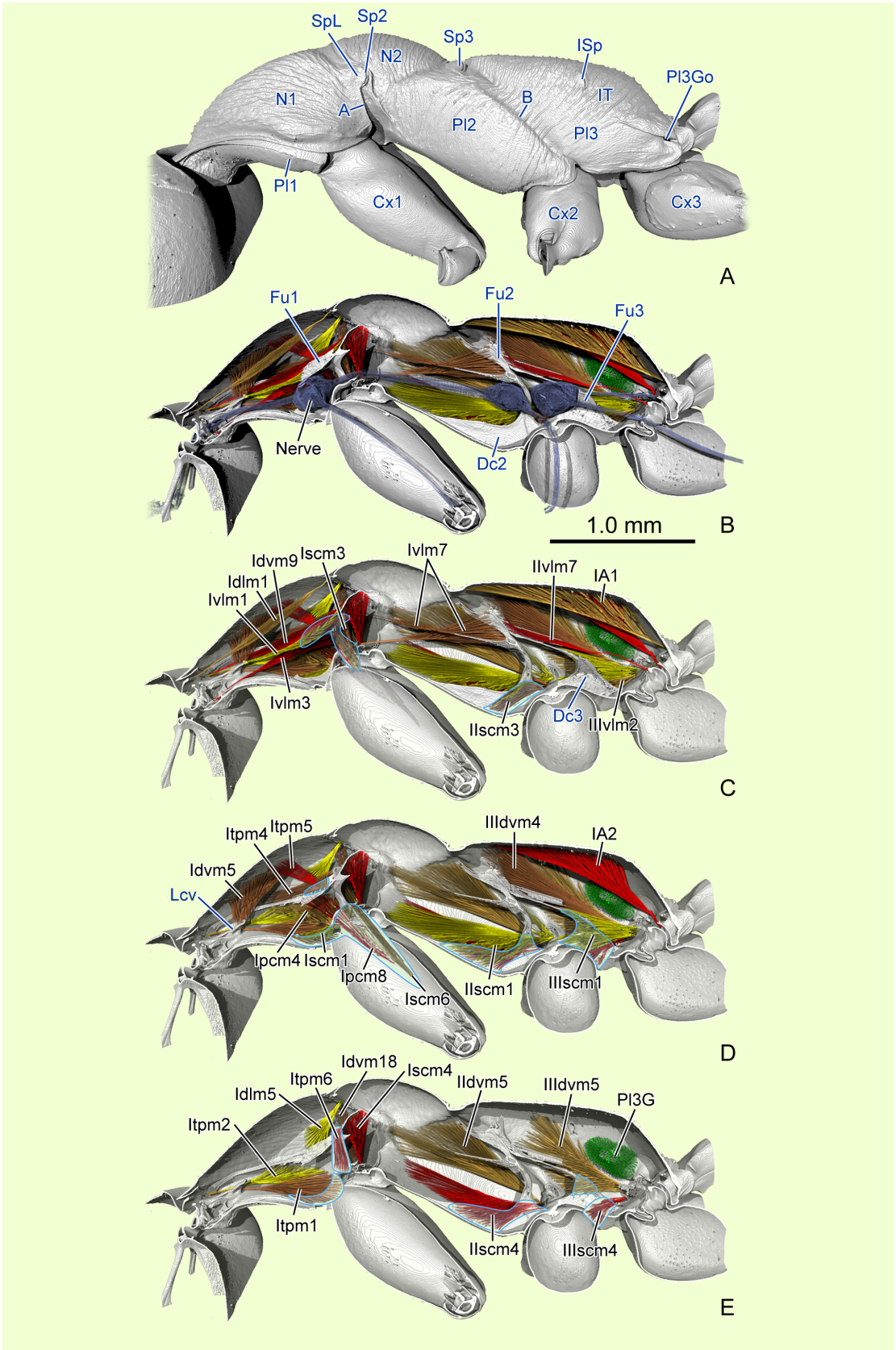

Fig. 4. *Myrmecia nigrocincta*, thorax, 3D reconstruction. **A**: lateral view of exoskeleton, **B–E**: endoskeleton and muscles, muscles removed layer by layer from median plane to lateral body wall.

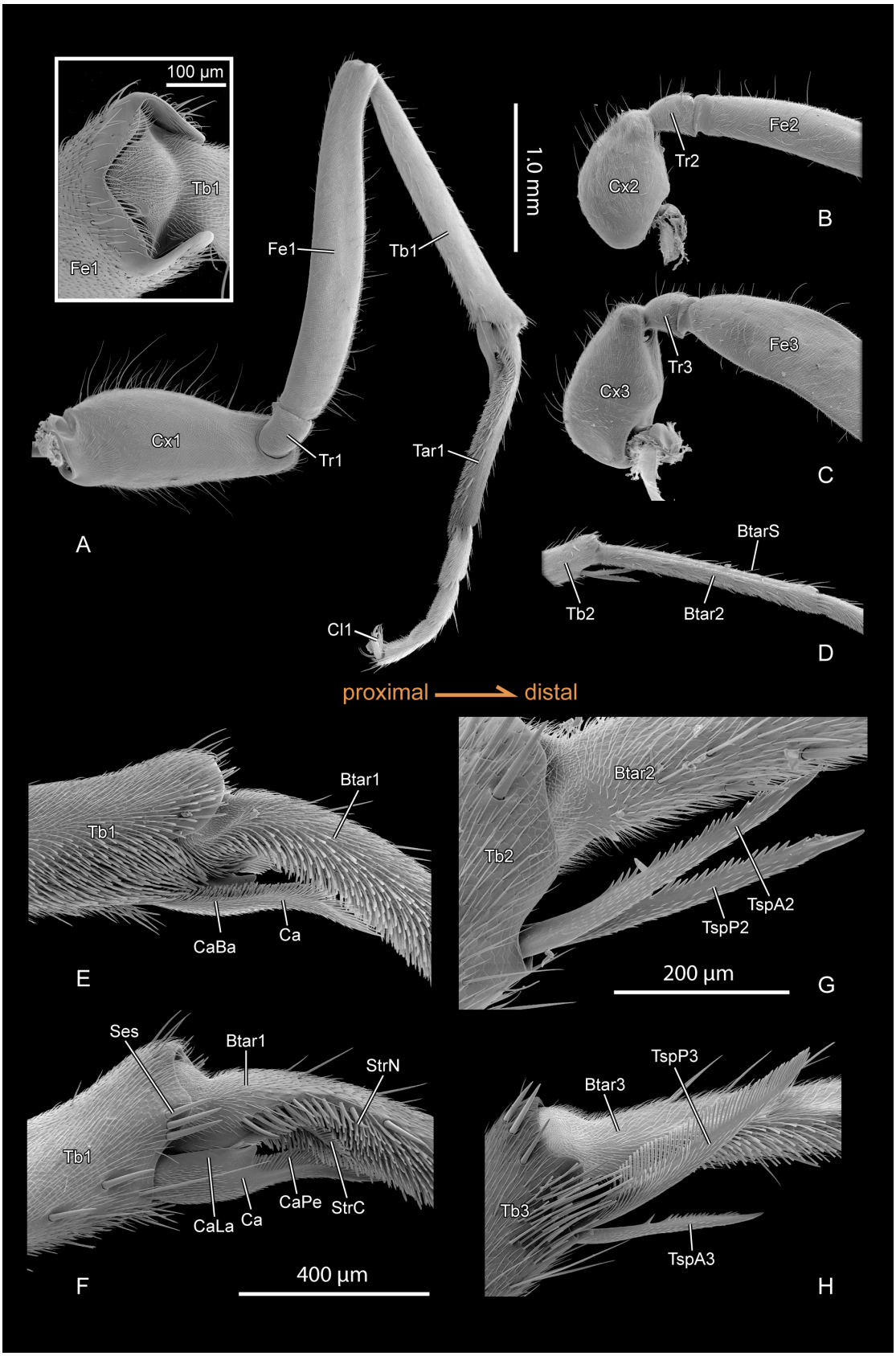

Fig. 5. *Myrmecia nigrocincta*, legs, SEM micrographs. **A**: foreleg, posterior view, insert shows femorotibial articulation in dorsal view, **B**: midleg, proximal part, posterior view, **C**: hindleg, proximal part, posterior view, **D**: tip of mesotibia and

mesobasitarsus with basitarsal sulcus, front view; **E**: strigil of foreleg, front view; **F**: strigil of foreleg, posterior view; **G**: mesotibial spurs of midleg, front view; **H**: metatibial spurs of hindleg, posterior view. Scale bars: 1.0 mm for A–D; 100 µm for the insert of A; 400 µm for E,F,H; 200 µm for G. Orange arrow indicates direction from proximal to distal.

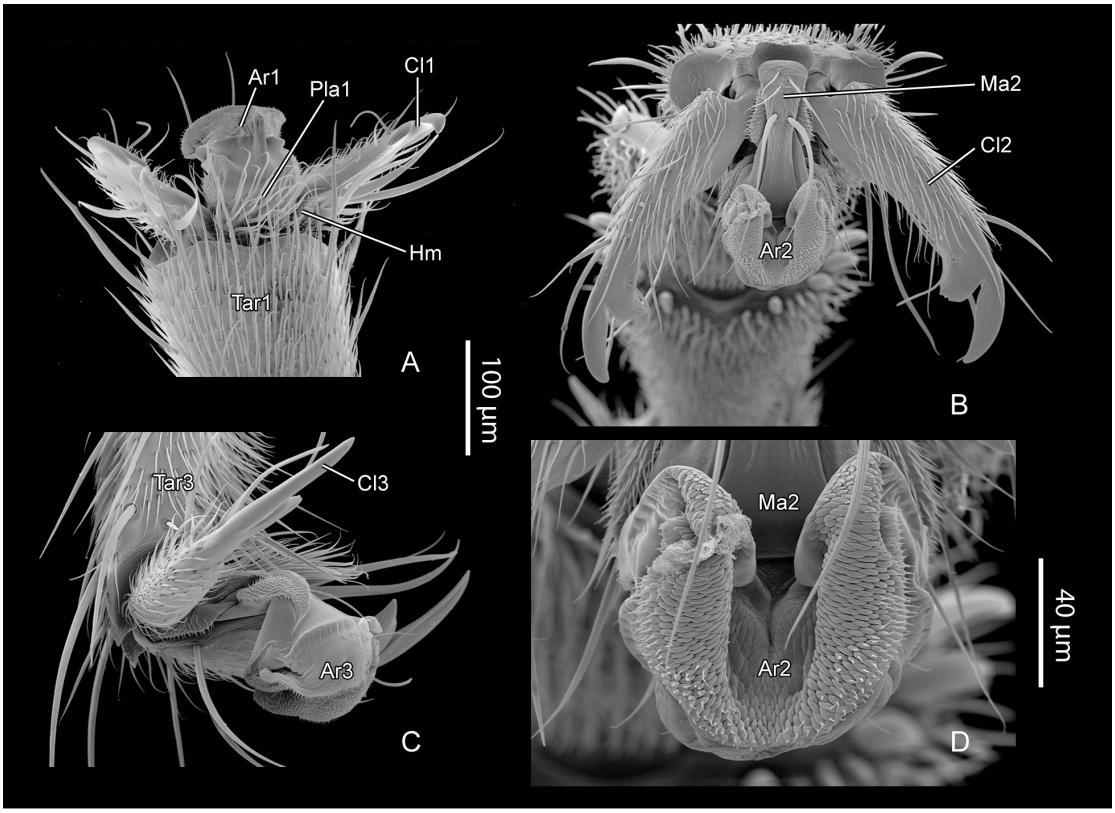

Fig. 6. *Myrmecia nigrocincta*, pretarsal structures, SEM micrographs. **A**: foreleg, dorsal view; **B**: midleg, front view; **C**: hindleg, lateral view; **D**: arolium, midleg. Scale bars: 100 µm for A–C; 40 µm for D.

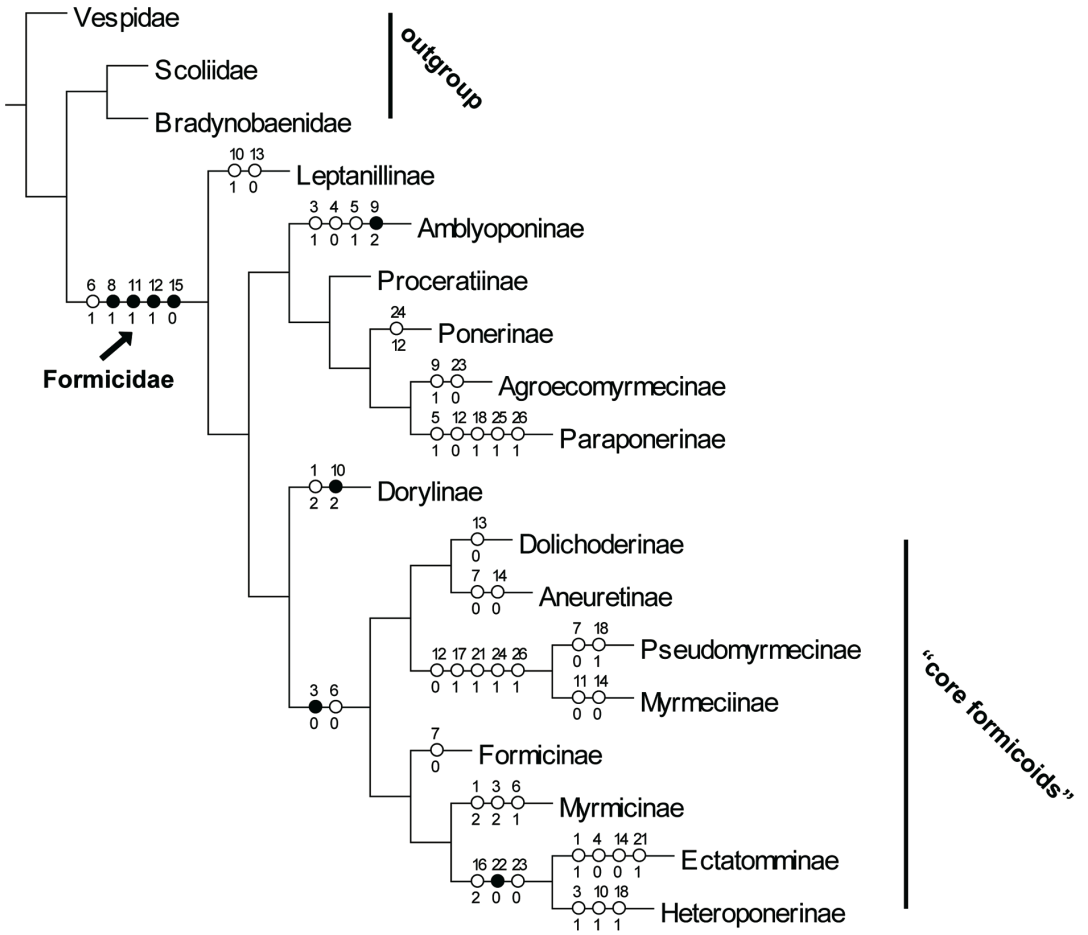

Fig. 7. Cladogram showing evolution of 26 thoracic skeletomuscular characters, based on molecular phylogeny of BRANSTETTER et al. (2017a). Apomorphies mapped on the tree as circles (full circles non-homoplasious changes). Character numbers are labeled above each circle, characters state numbers below.

3.5. Study V

Beutel, R.G., Pohl, H.W., Yan, E.V., Anton, E., **Liu, S.-P.**, Ślipiński, A., McKenna, D. & Friedrich, F. (2018)

The phylogeny of Coleopterida (Hexapoda) – morphological characters and molecular phylogenies. *Systematic Entomology*, (DOI: 10.1111/syen.12316).

Abstract

This manuscript presents a large morphological data set comprising external and internal features of adults and immature stages of Coleopterida and analyzes phylogenetically. A sister group relationship between Strepsiptera and monophyletic Coleoptera is supported by parsimony analyses. Archostemata is recovered as sister group of the remaining extant Coleoptera, and Polyphaga as sister group of Myxophaga. The heavy sclerotization without exposed membranes and a simplification of thoracic musculature are important characters of Coleoptera. The further simplifications of the thoracic locomotor apparatus are present in non-archostematan beetles. The subgroups of Coleopterida remain a challenge with parsimony and Bayesian analyses or morphological and molecular data suggesting different patterns. Meanwhile, the exploration of the earliest evolution in the fossil record is important to evaluating the relationships among beetle suborders.

3.5.1. Reviewed Version

The phylogeny of Coleopterida (Hexapoda) – morphological characters and molecular phylogenies

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Abstract. Coleopterida (Coleoptera + Strepsiptera) has been established as the sister group of Neuropterida (Megaloptera + Neuroptera + Raphidioptera) based on recent phylogenetic analyses of DNA sequence data obtained from genomes and transcriptomes. However, within the resulting clade (Neuropteroidea) the proposed sister group relationship between the highly specialized endoparasitic Strepsiptera and the megadiverse Coleoptera still lacks convincing morphological support. Furthermore, relationships among the four suborders of Coleoptera remain controversial, with morphological characters strongly conflicting with results suggested by molecular evidence. A large morphological data set comprising external and internal features of adults and immature stages is presented here and analyzed phylogenetically. Our study is focussed on deep splits in Coleopterida and on reconstructing character evolution on the phenotypic level. Parsimony analyses clearly support a sister group relationship between Strepsiptera and monophyletic Coleoptera. Presumptive synapomorphies are characters linked with posteromotorism, but also features of the head and prothorax. We recover Archostemata as sister group of the remaining extant Coleoptera, and Polyphaga as sister group of the species-poor suborder Myxophaga. The most important character complex of Coleoptera is heavy sclerotization without exposed membranes and a simplification of the thoracic muscle apparatus. Non-archostematan beetles are characterized by further simplifications of the thoracic locomotor apparatus. This trend reaches its peak in Myxophaga and Polyphaga, and these suborders also share apomorphies of the larval legs. A pattern with Polyphaga as sister to all other suborders and a clade Myxophaga + Archostemata (as in recent molecular phylogenetic studies) requires ten additional steps with our data set. This scenario implies that various simplifications of the thoracic exoskeleton and musculature have taken place several times independently, and also that a complex feeding apparatus suitable for saprophagy and sporophagy was ancestral in Coleoptera, with secondary reduction (or modification) in Archostemata and Adephaga). The coleopteran subordinal relationships remain a

challenge, with morphological and molecular data suggesting distinctly different patterns. The earliest evolution of Coleopterida is not documented in the fossil record. The exploration of potential stem-group fossils is a high priority, as is the study of species from the Permian-Triassic transition zone, which are apparently important in the context of evaluating the relationships among beetle suborders.

Introduction

With about 350,000 – 400,000 described extant species, beetles comprise roughly 25% of the diversity of all organisms (Stork *et al.*, 2015). In contrast, the highly specialized endoparasitic Strepsiptera (Fig. 1) are a very small group, with only slightly more than 600 known extant and extinct species (e.g. Kinzelbach, 1971; Pohl & Beutel, 2008). A close relationship between these two holometabolous orders was suggested before molecular approaches were used, with Strepsiptera proposed either as the sister taxon of monophyletic Coleoptera (Kinzelbach, 1971; Beutel & Gorb, 2001) or as a subordinate and secondarily highly modified polyphagan subgroup (Crowson, 1955, 1981). Some molecular phylogenetic analyses based exclusively on parsimony analyses of ribosomal DNA sequences suggested a group including Strepsiptera and Diptera (Halteria) (Whiting *et al.*, 1997; Wheeler *et al.*, 2001). This concept has not been supported by recent investigations based on morphology (Friedrich *et al.*, 2010; Beutel *et al.*, 2011), single copy nuclear genes (Wiegmann *et al.*, 2009; McKenna & Farrell, 2010; Ishiwata *et al.*, 2011), genomes (Niehuis *et al.*, 2012; see also McKenna, 2014), and transcriptomes (Peters *et al.*, 2014; Misof *et al.*, 2014; Boussau *et al.*, 2014). Monophyletic Coleoptera as the sister taxon of Strepsiptera is supported by most recent molecular phylogenetic studies (e.g. Peters *et al.*, 2014; Misof *et al.*, 2014; Boussau *et al.*, 2014; McKenna *et al.*, 2015). However, Coleopterida was only vaguely supported by morphological arguments so far, with most or all potential synapomorphies related to a single character complex, posteromotorism (Friedrich *et al.*, 2010; Beutel *et al.*, 2011). Moreover, character transformations in the exceedingly heterogenous Coleopterida remained obscure. The crucial issue of the basal splitting events in Coleoptera is not convincingly solved yet (McKenna, 2016; Beutel & McKenna, 2016), with either Archostemata (Beutel & Haas, 2000; Beutel *et al.*, 2008; Friedrich *et al.*, 2009) or Polyphaga (Kukalová-Peck & Lawrence, 1993, 2004; Misof *et al.*, 2014; McKenna *et al.*, 2015) placed as the sister group of the remaining suborders. A clade comprising the small suborders Archostemata and Myxophaga was recovered in recent analyses of molecular data (McKenna *et al.*, 2015), despite lacking morphological synapomorphies and striking differences in their habitus and life style (e.g. Reichardt, 1973; Hörnschemeyer, 2005).

Investigations of the morphology and phylogeny of the neuropteroid branch of Holometabola (Neuropterida, Strepsiptera, Coleoptera) have made impressive progress in the last two decades. This has been facilitated by new technologies accelerating the acquisition of high quality anatomical data (Friedrich *et al.*, 2013;

Wipfler *et al.*, 2016). Numerous studies were published on different life stages and body regions of Neuropterida (Aspöck & Aspöck, 2008; Beutel *et al.*, 2008; Beutel *et al.*, 2010a, b), Coleoptera (Anton & Beutel, 2004, 2006, 2012; Beutel *et al.*, 2008; Friedrich *et al.*, 2009; Beutel & Komarek, 2004; Friedrich & Beutel, 2006; Lawrence *et al.*, 2011; Anton *et al.*, 2016; Antunes-Carvalho *et al.*, 2017) and Strepsiptera (Beutel & Pohl, 2005; Osswald *et al.*, 2009; Koeth *et al.*, 2012; Fraulob *et al.*, 2015; Knauthe *et al.*, 2015; Pohl & Beutel, 2005, 2008). Moreover, extensive morphological data sets were compiled for the entire Holometabola (Friedrich & Beutel, 2010; Beutel *et al.*, 2011). Considering the wealth of morphological information available now and new phylogenetic results based on molecular data, it appeared appropriate to extend and improve previously analyzed data matrixes (Beutel & Haas, 2000; Beutel *et al.*, 2008; Friedrich *et al.*, 2008). The compiled data set of 190 characters of immature stages and adults was analyzed with maximum parsimony and Bayesian inference. The results are discussed with respect to recent phylogenies based on 8 single genes (Wiegmann *et al.*, 2009) and transcriptomes (Misof *et al.*, 2014). Possible scenarios of character evolution are evaluated under different phylogenetic patterns. The fossil recorded is discussed with respect to Coleopterida and early splitting events among beetles.

Methods

Taxon sampling

The taxon sampling is similar to that of Friedrich *et al.* (2009), but with the addition of Nevrorthidae (Neuroptera), *Lepicerus* Motschulsky (Lepiceridae, Myxophaga), and two terminals of Strepsiptera, *Xenos* Rossius and *Mengenilla* Hofeneder, the former representing a species rich family and the latter Mengenillidae, a family mostly characterized by plesiomorphic features (Pohl & Beutel, 2005, 2008). *Micromalthus* LeConte was excluded as a specialized member of Archostemata with many autapomorphies, but features of this unusual taxon (e.g. Hörnschemeyer, 2005) are provided in the list of characters. Specimens, SEM-micrographs, microtome sections and μ-CT data sets of species listed in (Friedrich *et al.*, 2009) were used for this contribution.

The core of the morphological character set is based on studies of Beutel & Haas (2000), Beutel *et al.* (2008) and Friedrich *et al.* (2009). Additional data were extracted from recent contributions on outgroup and ingroup taxa, including an extensive and well-documented data set for the entire Coleoptera (Lawrence *et al.*, 2011), investigations on the wing venation (Kukalová-Peck & Lawrence, 1993, 2004), and studies on Neuropterida (Beutel *et al.*, 2010a, b; Zimmermann *et al.*, 2011; Randolf *et al.* 2014), the cephalic morphology of Coleoptera (Dressler & Beutel, 2010; Anton *et al.*, 2016, Antunes-Carvalho, 2017), and the anatomy of the thorax (Friedrich & Beutel, 2006; Friedrich *et al.*, 2009; Koeth et al., 2012). More detailed information on selected taxa, fixation and applied techniques is provided in previous

studies (Friedrich *et al.*, 2009; Friedrich & Beutel, 2010; Beutel *et al.*, 2011; see also Wipfler et al., 2016 for a review of techniques).

Cladistic analysis

Data were entered in a matrix using WinClada (Nixon, 1999-2002). Parsimony analyses (all characters with equal weight and unordered) were carried out with NONA (ratchet, 1000 repl.) (Goloboff, 1995) and TNT (traditional search, random seed 1, 10 repl., TBR, 10 trees saved per repl.) (Goloboff *et al.*, 2008). Bremer-support values (Bremer, 1994) were calculated with NONA. *Zorotypus* Silvestri (Zoraptera), Raphidiidae, *Myrmeleon* Linnaeus sp., *Nevrorthus* Costa sp., Corydalinae, and *Sialis* Latreille sp. were used as outgroup taxa and treated as all other groups in the analysis (simultaneous analysis; Nixon & Carpenter, 1993). Bayesian analyses were performed with MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003, see also Lewis, 2001). The standard model for variable morphological characters (Mkv model; Ronquist & Huelsenbeck, 2003) was used with gamma for state frequencies and 0.1 for temperature. Four simultaneous runs of 5 million generations were conducted, each with one cold and three heated chains. Samples were drawn every 500 Markov chain Monte Carlo (MCMC) steps, with the first 25% discarded as burn-in. The run was automatically stopped when the average standard deviation of split frequencies was below 0.01. The analyses were carried out with a full data set of 190 characters and with a reduced set with 34 characters excluded, all of them presumptive autapomorphies of Strepsiptera (see Figs. A1, A2).

List of morphological characters

The names and numbers of the cephalic muscles were taken from v. Kéler (1963) and Dressler & Beutel (2010). Numbers for thoracic muscles are based on Beutel & Haas (2000) (and homologized with a generalized muscle list for Neoptera; Friedrich & Beutel, 2008) (see also additional file 1 and Beutel *et al.*, 2014).

The data matrices in Nexus format are provided as electronic supplements (see additional files 2, 3): Coleopterida_190char.nex (full data set with 190 characters), Coleopterida_156char.nex (reduced dataset with 34 characters (presumptive autapomorphies of Strepsiptera) removed).

Larvae, general

1. Size of 1st instar: (0) more than 0.3 mm; (1) less than 0.3 mm. Primary larvae of Strepsiptera (1st instar) are on average ca. 0.2 mm long and the minimum length is 0.07 mm (Pohl, 2000; Knauthe *et al.*, 2016) (Fig. 1A). A very small size can also be assumed for primary larvae of *Sphaerius*, but it is unlikely that they reach a similar degree of miniaturization. The penultimate instars are 0.84-1.20 mm long according to Britton (64) (see also (65: fig. 6.6D)) (coded as 0). Primary larvae of the endoparasitic Rhipiphoridae are also larger than those of Strepsiptera (66) and far-reaching effects

of miniaturization described for Strepsptera (Knauthe *et al.*, 2016) are unknown in Coleoptera (28-30).

Larvae, head

2. Dorsal endocarina: (0) absent; (1) present. Well developed in Archostemata (Beutel & Hörnschemeyer, 2002a, b) and some groups of Polyphaga not included here (Lawrence *et al.*, 2011). The occurrence in larvae of some scattered polyphagan families is apparently the result of parallel evolution. The endocarina is generally lacking in Scirtoidea and Staphyliniformia (Beutel & Leschen, 2005; Lawrence *et al.*, 2011).

3. Larval tentorium: (0) present; (1) absent. Completely reduced in larvae of Strepsiptera (Pohl, 2000; Müller, 2013; Pohl & Beutel, 2013; Knauthe *et al.*, 2016).

4. Caudal tentorial arms: (0) absent or short; (1) elongated, attached to posteroventral part of head capsule. Elongated and posteriorly connected with head capsule in Dytiscoidea excl. Noteridae (Beutel, 1993; Beutel *et al.*, 2013). Coded as inapplicable for Strepsiptera.

5. Orientation of head: (0) subprognathous, slightly to moderately inclined; (1) prognathous or hyperprognathous; (2) hypognathous. Prognathous in Adephaga (Beutel, 1993) and Archostemata (Beutel & Hörnschemeyer, 2002a, b), and also in Hydrophiloidea (excl. Spercheidae (Beutel, 1999) and some other groups of Polyphaga (Elateroidea, Cantharoidea, Cleroidea) (Beutel, 1995; Beutel & Pollock, 2000). Hypognathous in Scarabaeoidea and most groups of Chrysomeloidea (Lawrence *et al.*, 2011; Scholtz & Grebennikov, 2016).

6. Head shape of later instars: (0) not transverse, not strongly rounded laterally; (1) transverse, broader than long, strongly rounded laterally; (2) globular and simplified. Transverse in later instars of Archostemata (Beutel & Hörnschemeyer, 2002a, b) and Myxophaga (Beutel *et al.*, 1999), even though less distinctly in the presumptive larva of *Lepicerus* (Lawrence *et al.*, 2013). Also it is transverse in some Scirtidae (LeSage, 1991; Lawrence, 2016), coded as 0&1). Globular and strongly simplified in endoparasitic secondary larvae of Strepsiptera (Müller, 2013).

7. Deep dorsal and ventral posteromedian emargination: (0) absent; (1) present. Present in Archostemata (Beutel & Hörnschemeyer, 2002a, b).

8. Hemispherical projection of head capsule between mandibular and maxillary articulation: (0) absent; (1) present. Present in Hydraenidae, Leiodidae, Agyrtidae and Ptiliidae (Beutel & Leschen, 2005).

9. Posterior tentorial grooves: (0) close to hind margin of head; (1) shifted anteriorly. Distinctly shifted anteriorly in Nevrorthidae, Raphidioptera, Corydalidae (Beutel & Friedrich, 2008), and several groups of Coleoptera, especially in Adephaga, Hydrophiloidea and Staphylininae (Beutel, 1993, Beutel, 1999; Beutel & Molenda, 1997).

10. Gula: (0) absent or not recognisable as a defined sclerotized element; (1) undivided sclerotized quadrangular gula; (2) strongly narrowed gula. Well-developed

and undivided larval gula present in Raphidioptera, Corydalidae (Beutel & Friedrich, 2008), Nevrorthidae (Beutel *et al.*, 2010a), and Coleoptera (partim; Beutel & Hörnschemeyer, 2002a, b). Strongly narrowed in some groups of Adephaga (Gyrinidae, Trachypachidae, Carabidae major part) and most groups of Hydrophiloidea (Beutel, 1993, 1999). The short and indistinctly defined gula of *Zorotypus* is scored as 0. The posteroventral sclerite of the head capsule of larval Nevrorthidae is a gula in terms of position and specific quality (Beutel *et al.*, 2010a), even though recent results of molecular studies (Wang *et al.*, 2017; Winterton *et al.*, 2018) suggest that it may have evolved independently and is a potential autapomorphy the family.

11. Number of retinula cells in ommatidia or stemmata: (0) less than 15; (1) 15 or more. Number strongly increased in Neuropterida, especially in Megaloptera where up to 40 retinula cells can be present (Paulus, 1986).

12. Articulation of labrum: (0) free; (1) partly fused; (2) completely fused. Fused in Adephaga and Hydrophiloidea (Beutel, 1993, 1999), and also in some other groups of Polyphaga (e.g. Cantharidae; Beutel, 1995). Partly fused in Dascillidae (Lawrence *et al.*, 2011).

13. Anterior margin of head capsule; (0) not forming sharp cutting edge; (1) forming sharp cutting edge. The wedge-shaped head of primary larvae of Strepsiptera is characterized by a sharp anterior cutting edge (Knauthe *et al.*, 2016) (Fig. 1A). A rounded or toothed anterior margin of the clypeolabrum occurs in some groups of beetles with prognathous and predacious larvae (e.g. Adephaga, Hydrophiloidea; (Beutel, 1993, 1999). However, it does not form a cutting edge suitable for penetrating the body wall of an insect.

14. M. frontolabralis (M. 8) of later instars: (0) present; (1) absent. Present in *Raphidia* (Beutel & Ge, 2007) and Megaloptera (Röber, 1942; Beutel & Friedrich, 2008), but absent in Neuroptera (Beutel *et al.*, 2010a) and Strepsiptera (Pohl, 2000; Knauthe *et al.*, 2016). Also absent in Coleoptera (Beutel & Hörnschemeyer, 2002a, b, Beutel, 1993, 1999; Beutel & Molenda, 1997) with the exception of 1st instar larvae of *Tenomerga* (Yavorskaya *et al.*, 2015) (scored as 1 for Cupedidae).

15. M. frontoepipharyngalis (M. 9): (0) present; (1) absent. Present in some groups of Coleoptera (e.g. Cupedidae, Myxophaga excl. Hydroscaphidae; (Das, 1937; Beutel & Hörnschemeyer, 2002a, b; Beutel *et al.*, 1999; Yavorskaya *et al.*, 2015), in *Raphidia* Linnaeus (Beutel & Ge, 2007) and Megaloptera (Röber, 1942; Beutel & Friedrich, 2008), and in some genera of Neuroptera (Wundt, 1961; Rousset, 1966; Beutel *et al.*, 2010a). Absent in Strepsiptera (Knauthe *et al.*, 2016).

16. Antenna of primary larva: (0) distinctly developed; (1) reduced. Reduced in larvae of Strepsiptera, only recognizable as an entirely flat antennal field (Knauthe *et al.*, 2016) (Fig. 1A).

17. Number of antennomeres in last instar: (0) more than thirteen; (1) five; (2) four; (3) three; (4) two; (5) no antennomere recognizable. Multi-segmented in larvae of Scirtidae (LeSage, 1991; Lawrence, 2016) and also in some larvae of Neuroptera,

possibly related to a secondary subdivision of the penultimate antennomere (Beutel *et al.*, 2010a, coded as 0). Four-segmented in Sialidae (Röber, 1942), Raphidioptera (Beutel & Ge, 2007), Cupedidae and Ommatidae (Lawrence, 1982, 1999; Beutel & Hörnschemeyer, 2002a, b), in Adephaga (Beutel, 1993) and in few groups of Polyphaga (Lawrence, 1982). Five-segmented in Corydalidae (Beutel & Friedrich, 2008) and usually also in scarabaeoid larvae (Lawrence *et al.*, 2011). Three-segmented in Chrysopidae (Beutel *et al.*, 2010a), Lepiceridae (Lawrence *et al.*, 2013) and almost generally in Polyphaga (Lawrence *et al.*, 2011). Two-segmented in Myxophaga excl. Lepiceridae and in 1st instar larvae of *Tenomerga* Neboiss (Beutel *et al.*, 1999, Yavorskaya *et al.*, 2015). No recognizable antennomeres are present in secondary larvae of Strepsiptera (Pohl, 2000; Knauthe *et al.*, 2016).

18. Sensorium on antepenultimate antennomere: (0) absent; (1) present. Present in larvae of Megaloptera (Beutel & Friedrich, 2008). Not applicable in Strepsiptera.

19. Exposure of mouthparts: (0) exposed; (1) semi-entognathous, labrum laterally fused with triangular genal lobe; (2) mandibles largely internalized, ventral mouthparts partly fused with head capsule. Semi-entognathous in Hydroscaphidae and Sphaeriusidae (Beutel *et al.*, 1999). Mandibles of primary strepsipteran larvae largely internalized, ventral mouthparts strongly modified (Pohl, 2000; Knauthe *et al.*, 2016).

20. Mandibular apex: (0) slender, with one or several pointed teeth; (1) three blunt and strong teeth; (2) blunt, with more than three apical teeth. With three strong apical teeth in Archostemata (Beutel & Hörnschemeyer, 2002a, b; Yavorskaya *et al.*, 2015). More than three teeth in many chrysomelid larvae (e.g. (Lawrence *et al.*, 2011).

21. Mandibular mola: (0) present, not quadrangular, not delimited by distinct margin; (1) present, quadrangular, delimited by distinct margin; (2) absent. Present in Archostemata (Beutel & Hörnschemeyer, 2002a, b; Yavorskaya *et al.*, 2015), Myxophaga (Beutel *et al.*, 1999), and many groups of Polyphaga (e.g. Scirtoidea) (Lawrence, 2016). Quadrangular and delimited by a distinct margin in older instars of Cupedidae (coded as 1) and *Micromalthus* (Beutel & Hörnschemeyer, 2002a, b).

22. Prostheca: (0) absent; (1) present, rounded and semimembranous; (2) present, slender. Rounded and semimembranous, with small, posteriorly directed spines in Torridincolidae and Hydroscaphidae (Beutel *et al.*, 1999). Slender, with one or several apices in Hydraenidae, Agyrtidae, Leiodidae (partim), Clambidae, Eucinetidae (coded as absent for Scirtidae), Derodontidae, and others (Lawrence, 1982; 2016; Beutel & Leschen, 2005; Lawrence *et al.*, 2011).

23. Accessory ventral process of mandible: (0) absent; (1) present. Present in Clambidae (partim), Scirtidae, Dascillidae, Scarabaeoidea and Derodontidae (Lawrence, 2016; Lawrence *et al.*, 2011).

24. Intramaxillary movability: (0) fully retained; (1) reduced, not foming fuctional complex with labium; (2) reduced, maxillolabial complex; (3) Maxillae medially connected. Movability distinctly reduced in Raphidioptera, Adephaga (excl. Gyrinidae), Hydrophiloidea, and Histeroidea (Beutel & Haas, 2000; Beutel, 1993, 1999). Maxillolabial complex present in most groups of Elateriformia (Beutel, 1995)

and Cleroidea (Beutel & Pollock, 2000). Medially connected and plate-like in primary larvae of Strepsiptera (Pohl, 2000, Knauthe *et al.*, 2016). Coded as inapplicable for Neuroptera (see char. 25) (Beutel *et al.*, 2010a).

25. Link between mandibles and maxillae: (0): absent; (1) maxillae form sucking jaws with mandibles. The formation of sucking jaws by the longitudinal interlocking of the mandibles and maxillae is a unique feature of Neuroptera (Beutel *et al.*, 2010a).

26. Cardo: (0) not subdivided into several sclerites; (1) subdivided into several sclerites. Subdivided in Hydrophiloidea (Beutel, 1999). Coded as inapplicable for Strepsiptera where a cardo is not present as a defined maxillary element (Knauthe *et al.*, 2016).

27. Separate galea: (0) present; (1) absent. Not present as separate element in Myxophaga, in most subgroups of Staphylinidae, in Clambidae (with the exception of *Calyptomerus* Redtenbacher; coded as 0), and in Cucujiformia (Lawrence, 1982, 2016; Lawrence *et al.*, 2011). Probably forming a mala with the lacinia in these groups. Missing in *Hygrobia* and Strepsiptera (Pohl, 2000; Beutel *et al.*, 2006).

28. Insertion of galea: (0) stipes or unsclerotized proximomesal part of palpifer 1; (1) distal part of palpifer. Inserted on distal part of palpifer in Hydrophiloidea (Beutel, 1999).

29. Maxillary palp: (0) present and composed of several segments: (1) distinctly reduced, bolt-shaped socket and long seta; (2) absent. Maxillary palp and palp muscles absent in Neuroptera (Beutel *et al.*, 2010a). Composed of bolt-shaped socket and long seta in primary larvae of Strepsiptera (Knauthe *et al.*, 2016).

30. Submentum and mentum: (0) not fused and narrowed between maxillary fossae; (2) fused and narrowed between maxillary fossae. Fused and narrowed between maxillary fossae in larvae of Archostemata (Beutel & Hörnschemeyer, 2002a, b; Yavorskaya *et al.*, 2015). Ventral mouthparts highly modified in primary larvae of Strepsiptera (coded as inapplicable).

31. Ligula: (0) not wedge-shaped and enlarged; (1) enlarged and wedge-shaped. Enlarged, sclerotized, and wedge-shaped in Archostemata (Beutel & Hörnschemeyer, 2002a, b; Yavorskaya *et al.*, 2015).

32. M. submentopraementalis (M. 28): (0) present; (1) absent. M. submentopraementalis is absent in primary larvae of Strepsiptera (Knauthe *et al.*, 2016), in larvae of Archostemata (Beutel & Hörnschemeyer, 2002a, b; Yavorskaya *et al.*, 2015), and in some other groups of beetles (Beutel, 1993). It is generally present in Neuropterida (e.g. *Chrysopa* Leach, *Sialis*; Röber, 1942; Wundt, 1961; Beutel & Friedrich, 2008) and also in most groups of Coleoptera (Beutel, 1993, 1995, 1999).

33. External opening of preoral cavity: (0) single opening (functional mouth opening); (1) double opening. Double opening present in primary larvae of Strepsiptera (Pohl, 2000; Knauthe *et al.*, 2016).

34. Larval salivary ducts and glands: (0) well developed; (1) strongly narrowed, without recognizable lumen; (2) absent; (3) tube-like elongated glands without ducts. Narrow, vestigial proximal salivary tube present in Megaloptera (Beutel & Friedrich,

2008). Absent in Nevrorthidae (Beutel *et al.*, 2010a), non-cucujiform Coleoptera (Beutel, 1993, 1995, 1999; Beutel & Hörnschemeyer, 2002a, b, Beutel & Molenda, 1997; Beutel *et al.*, 1999; Yavorskaya *et al.*, 2015) and Strepsiptera (Knauthe *et al.*, 2016). Long tube-like glands are present in Cucujiformia (e.g. Beutel & Pollock, 2000). Their structure is completely different from salivary glands found in other groups of insects.

35. Position of brain: (0) head or anterior prothorax; (1) middle region of posterior postcephalic body. Brain and suboesophageal ganglion shifted to middle region of postcephalic body in primary larvae of Strepsiptera (Beutel *et al.*, 2005).

Larvae, thorax

36. Cervix: (0) absent; (1) present. Distinct separate cervix present in Neuroptera (Beutel *et al.*, 2010a). Absent in all other groups.

37. Sternal plates: (0) absent; (1) present. Sternal plates formed by true sternites inserted between coxae in all primary larvae of Strepsiptera (Pohl, 2000; Osswald *et al.*, 2009).

38. Leg segmentation: (0) six free leg segments; (1) tibia fused with tarsus; (2). Five, femur fused with trochanter. Six-segmented in Adephaga and Archostemata with well-developed legs (Lawrence, 1982, 1999; Beutel & Hörnschemeyer, 2002a, b). Five-segmented with tibiotarsus in Myrmeleontidae (Sundermeier, 1940), Myxophaga and Polyphaga (Lawrence, 1982; Beutel *et al.*, 1999). Femur fused with tibia in primary larvae of Strepsiptera (Fig. 1A), with a suture still recognizable in Mengenillidae (Pohl, 2000; Osswald *et al.*, 2009).

39. Number of claws: (0) double; (1) single. Single claw in Haliplidae, Myxophaga, Polyphaga, and first instar larvae of *Priacma* LeConte (coded as 0&1 for Cupedidae) (Lawrence, 1999; Beutel & Hörnschemeyer, 2002a, b; Yavorskaya *et al.*, 2015). Claws absent in Strepsiptera (coded as inapplicable) (Pohl, 2000; Pohl & Beutel, 2004).

40. Lobe-like pretarsal attachment pads: (0) absent; (1) present. Present in primary larvae of Strepsiptera (Pohl, 2000; Pohl & Beutel, 2004), differing distinctly from adhesive devices occurring in larvae of very few groups of Coleoptera (Coccinellidae; coded as 0).

41. Regular rows of lancet-shaped setae on hind margins of thoracic and abdominal terga: (0) absent; (1) present. Present in Hydroscaphidae and Sphaeriusidae (Beutel *et al.*, 1999).

Larvae, abdomen

42. Posterior edges of abdominal sternites: (0) not densely covered with bristles and spinulae; (1) with densely arranged bristles and spinulae. Dense rows of bristles and spinulae present on posterior edges of abdominal sternites of primary larvae of Strepsiptera (Pohl, 2000; Knauthe *et al.*, 2016).

43. Abdominal tergal ampullae: (0) absent; (1) present. Present in Archostemata (Beutel & Hörnschemeyer, 2002a, b; Yavorskaya *et al.*, 2015).
44. Setiferous lateral gill filaments: (0) absent; (1) present. Setiferous lateral tracheal gills are present in larvae of Megaloptera (Aspöck & Aspöck, 2003), Gyrinidae, *Coptotomus* (Dytiscidae), and *Berosus* (Hydrophilidae), and they also occur in a subgenus of *Helophorus* Leach (Helophoridae) (Lawrence *et al.*, 2011).
45. Spiracular gills: (0) absent; (1) present, long and slender; (2) present, short and bulbous. Present in Myxophaga excluding Lepiceridae (Beutel *et al.*, 1999; Lawrence *et al.*, 2013). Short and bulbous in Hydroscaphidae and Sphaeriusidae (Beutel *et al.*, 1999).
46. Abdominal segment IX: (0) well developed, tergum present; (1) largely reduced, tergum absent. Largely reduced in Dytiscoidea with the exception of Aspidytidae (Beutel *et al.*, 2006).
47. Abdominal segment XI: (0) well developed, tergum present; (1) absent. Present in primary larvae of Strepsiptera (Beutel *et al.*, 2011).
48. Size and position of spiracles VIII: (0) not enlarged and terminal; (1) enlarged and terminal. Enlarged in Noteridae, Amphizoidae, and Dytiscidae. Closed and replaced by ventral gills in Hygrobiidae (coded as 0) (Beutel *et al.*, 2006).
49. Urogomphi: (0) absent; (1) present, fixed; (2) present, articulated. Articulated or fixed urogomphi missing in Archostemata (Lawrence, 1982, 1999; Beutel & Hörnschemeyer, 2002a, b), Hydroscaphidae, and Sphaeriusidae, and also in many groups of Polyphaga (e.g. Scarabaeidae, Scirtoidea, Dascillidae) (Lawrence, 1982, 2016; Lawrence *et al.*, 2011). Movable urogomphi occur in Adephaga and Staphylinoidea (e.g. Beutel & Leschen, 2005; Lawrence *et al.*, 2011).
50. Terminal abdominal jumping apparatus of segment XI: (0) absent; (1) present. Formed by long and strongly developed bristles (cerci) of abdominal segment X in primary larvae of Strepsiptera (Pohl & Beutel, 2004). Jumping capacity secondarily reduced in *Stylops* Kirby (Kinzelbach, 1971).

Larval ecology
51. Larval habitat: (0) terrestrial or semiaquatic; (2) aquatic. Larvae of Nevrorthidae, Sisyridae and Megaloptera are aquatic (e.g. Beutel & Friedrich, 2008; Beutel *et al.*, 2010a; Randolf *et al.*, 2013) and also immatures of different groups of Coleoptera (e.g. Reichardt, 1973; Crowson, 1981; Lawrence, 1982). Larvae of Osmylidae are found in riparian habitats (coded as 0). Aquatic larval development apparently evolved independently in Adephaga (Gyrinidae, Haliplidae, Dytiscoidea), Myxophaga (Torridincolidae, Hydroscaphidae) and several polyphagan subgroups (e.g. Hydraenidae, Hydrophiloidea major part; Elmidae etc.) (Beutel, 1997).
52. Endoparasitism of larvae: (0) absent; (1) present. Immature stages of Strepsiptera are endoparasitic (e.g. Pohl & Beutel, 2005) (Fig. 1B).

Pupal characters

53. Movability of pupal mandible: (0) absent; (1) present. Pupal mandible movable in Neuropterida (Aspöck & Aspöck, 2003) and few other groups of Holometabola. Immobilized in Coleoptera and in contrast to Kinzelbach (1975) also in Strepsiptera.

Adults, general

54. Sclerites: (0) connected by extensive, externally exposed membranes; (1) firmly connected, no membranes exposed externally. Sclerites not covered by elytra closely attached to each other in Coleoptera (with few exceptions), without exposed membranes (Lawrence, 1982; Beutel & Haas, 2000) (Fig. 2).

55. Cuticular surface: (0) without scale-like structures: (1) scale-like structures present. Scale-like surface structures inserted on cuticular tubercles (Fig. 2A) in Cupedidae and Ommatidae, but not in *Micromalthus* and the other groups of Archostemata (Beutel *et al.*, 2008; Friedrich *et al.*, 2009).

56. Dense vestiture of microtrichia on cuticular surface: (0) absent; (1) present. Present in males of Strepsiptera (Pohl & Beutel, 2005) (Fig. 1G, H).

Adults, head

57. Orientation of head: (0) orthognathous; (1) prognathous or slightly inclined. Orthognathous in Neuroptera and Stylopidia (Fig. 1H), prognathous or slightly inclined in Coleoptera, Megaloptera, Raphidioptera and basal groups of Strepsiptera (Beutel *et al.*, 2008, 2010b, 2011). A variety of head positions has evolved in Polyphaga (e.g. Eucinetidae, Anobiidae, Mordellidae), apparently as results of independent evolution (Lawrence *et al.*, 2011).

58. Constricted neck and postocular extensions: (0) absent or indistinct; (1) present. Strongly constricted neck region and distinct postocular extensions present in Ommatidae and Cupedidae (Beutel *et al.*, 2008). Constriction also present in *Nicrophorus* Fabricius (Silphidae) (Lawrence *et al.*, 2011).

59. Shape of frontal region: (0) without V- or U-shaped impression; (1) V- or U-shaped impression present. V- or U-shaped impression present on frontal region of males of Strepsiptera excl. †Protoxenidae (Pohl & Beutel, 2005; Pohl *et al.*, 2005).

60. Dorsomedian longitudinal groove on head: (0) absent; (1) present. Present in Hydrophiloidea excl. Spercheidae (Anton & Beutel, 2004; Beutel & Leschen, 2005).

61. Gula: (0) absent; (1) present, membranous or weakly sclerotized and short; (2) present, sclerotized. Present and sclerotized in Coleoptera with very few exceptions (Lawrence *et al.*, 2011). Also present in Raphidioptera and Megaloptera (Röber, 1942; Beutel *et al.*, 2011). Due to the complete fusion of the ventral elements of the head including the labium the presence of a gula in Strepsiptera cannot be verified (coded as ?).

62. Functional and structural subdivision of compound eyes: (0) absent; (1) present. Morphologically and functionally completely divided in Gyrinidae, with a narrow separating chitinous bar in the groundplan and a broad interocular bridge in

Gyrininae (Beutel, 1989a; Beutel *et al.*, 2017). A fully divided compound eye also occurs in the hydrophilid genus *Amphiops* Erichson (Lawrence *et al.*, 2011).

63. Raspberry compound eyes: (0) absent; (1) present. Present in Strepsiptera (Fig. 1G, H), with large ommatidia separated by chitinous bridges densely set with microtrichia (Buschbeck *et al.*, 1999, 2003; Pohl & Beutel, 2005).

64. Ocelli: (0) three well-developed ocelli; (1) two small ocelli; (2) absent. Three well developed ocelli in Neuropterida excl. Inocellidae (Beutel *et al.*, 2011), two in few groups of Coleoptera (e.g. Hydraenidae partim, Agyrtidae partim (not in *Necrophilus* Latreille), Derodontidae partim, few Leiodidae), and one in some Dermestidae. Absent in most groups of beetles and in Strepsiptera (Leschen & Beutel, 2004; Pohl & Beutel, 2005).

65. M. frontolabralis (M. 8): (0) present, origin on frons; (1) absent. Absent in Coleoptera (e.g. Beutel *et al.*, 2008, 2011; Anton *et al.*, 2016, Antunes-Carvalho, 2017) and Strepsiptera (Pohl & Beutel, 2005). Present in Neuropterida (Röber, 1942; Achtelig, 1967; Beutel *et al.*, 2011; Randolf *et al.*, 2013, 2014; Zimmermann *et al.*, 2011).

66. Number of antennomeres: (0) more than thirteen; (1) eleven; (2) less than eleven. Multisegmented in Neuropterida (Beutel *et al.*, 2011) and other holometabolous orders. Eleven-segmented in most groups of Coleoptera (e.g. Archostemata, Dytiscoidea, Carabidae, Eucinetidae, Scirtidae, Derodontidae). Less than eleven in Strepsiptera (Fig. 1G, H), Gyrininae, Lepiceridae, Hydroscaphidae, Hydraenidae (partim), Hydrophiloidea, Clambidae, and Scarabaeidae (Anton *et al.*, 2016; Antunes-Carvalho *et al.*, 2017).

67. Antennal flabella: (0) absent; (1) present. Elongate flabella generally present in Strepsiptera (antennomeres 3-7 in the groundplan) (Pohl & Beutel, 2005; Pohl *et al.*, 2005).

68. Hofender's organ: (0) absent; (1) present. Present in males of Strepsiptera as specific sensorial groove of antennomere 4 (Pohl & Beutel, 2005; Pohl *et al.*, 2005).

69. Dense vestiture of dome-shaped antennal chemoreceptors: (0) absent; (1) present. Present in males of Strepsiptera (Pohl & Beutel, 2005; Pohl *et al.*, 2005).

70. Antennal club formed by densely pubescent three distal antennomeres: (0) absent or club formed by different number of segments or without breathing function; (1) present, symmetrical, used as accessory breathing organ; (2) present, strongly asymmetrical. Three-segmented pubescent club present and used as accessory breathing organ in Hydrophiloidea (e.g. Hansen, 1991) (coded as 0 for *Nicrophorus*). Club asymmetrical and mostly 3-segmented in Scarabaeoidea (Scholtz & Grebennikov, 2016). Five-segmented club present and used as accessory breathing organ in most groups of Hydraenidae (coded as 0).

71. Pedicellus (0) cylindrical, not ear-shaped; (1) enlarged, ear-shaped, with fringe of long hairs. Highly modified pedicellus functions as receptor of vibrations of the water surface in Gyrinidae (Beutel 1989a; Beutel *et al.*, 2017).

72. Mandibular mola: (0) absent; (1) present. Absent in Archostemata and Adephaga, and also missing in several groups of Polyphaga (e.g. Staphylinidae (partim), Scirtidae (partim), Eucinetidae (partim), Dascillidae, Scarabaeidae (partim), Elateroidea (partim), Elateroidea) (Lawrence *et al.*, 2011). Present in Myxophaga and in different polyphagan lineages such as Staphylinoidea (major part), Hydrophiloidea s.l. (absent in some histerids), Clambidae, Eucinetidae (major part), Scirtidae (partim), Byrrhidae, Derodontidae, Coccinellidae, Tenebrionidae, and Chrysomelidae (Lawrence *et al.*, 2011).

73. Single movable mandibular preapical tooth (prostheca): (0) absent; (1) present on left mandible. Present on left mandible in Myxophaga (excl. Sphaeriusidae) (Reichardt, 1973; Anton & Beutel, 2006; Lawrence *et al.*, 2011;) and Clambidae (Anton *et al.*, 2016). Similar tooth inserted on both mandibles of *Ochthebius* Leach (Hydraenidae) (coded as 0).

74. Galea: (0) present, not palp-like; (1) palp-like; (2) vestigial; (3) fused with lacinia; (4) completely absent. Palp-like and usually composed of two cylindrical smooth galeomeres in Adephaga (Dressler & Beutel, 2010; Lawrence *et al.*, 2011), but one-segmented in Amphizoidae, and one-segmented or absent in Gyrininae (Beutel 1989a; Beutel *et al.*, 2006, 2017; Lawrence *et al.*, 2011). Vestigial in *Micromalthus* and completely fused with lacinia in Myxophaga. Completely reduced in Strepsiptera excl. †*Protoxenos* Pohl, Beutel and Kinzelbach (Pohl & Beutel, 2005; Pohl *et al.*, 2005).

75. Maxillary palp: (0) composed of several palpomeres; (1) single palpomere. All palpomeres fused in Strepsiptera (Pohl & Beutel, 2005; Pohl *et al.*, 2005).

76. Muscle originating from lateral head capsule and inserting on membrane proximad maxillary base (M. craniobasimaxillaris s. Anton and Beutel, 2012): (0) absent; (1) present. Occurs in Staphyliniformia and Scarabaeoidea (e.g. Anton & Beutel, 2004, 2012; Antunes-Carvalho *et al.*, 2017), but is missing in *Nicrophorus* and *Cetonia* Fabricius (Scarabaeidae).

77. Prementum: (0) present as separate element; (1) absent, completely fused with other labial elements and head capsule. Not present as recognizable separate element in Strepsiptera (Pohl & Beutel, 2005). Labial palps, endite lobes and proximal labial elements also missing.

78. Anterolateral margin of mentum: (0) without rounded lobes; (1) rounded lobes present. Lobes present in Adephaga (e.g. Dressler & Beutel, 2010). Inapplicable in Strepsiptera.

79. Hypopharynx hourglass-shaped between paired mouthparts. Hypopharynx strongly narrowed between paired mouthparts in most groups of Staphyliniformia including Scarabaeoidea (Anton & Beutel, 2004, 2012). Broad in Silphidae and Scydmaeninae (Lawrence, 2016). Inapplicable in Strepsiptera.

80. Dorsal and ventral wall of preoral cavity: (0) with longitudinal epi- and hypopharyngeal bulges with fields of microtrichia; (1) without longitudinal epi- and hypopharyngeal bulges with fields of microtrichia; (2) longitudinal bulges replaced by

a antepipharyngeal-prelabial complex. Feeding apparatus with hairy longitudinal epi- and hypopharyngeal bulges present in Myxophaga (e.g. *Lepicerus*, *Satonius* (Endrödy-Younga); Anton & Beutel, 2006) and many groups of Polyphaga (Anton & Beutel, 2004, 2012; Lawrence *et al.*, 2011). Fields of microtrichia on longitudinal bulges interact with brushes on proximal parts of mandibles, likely in correlation with microphagous feeding habits. In all examined species of Elateroidea s.l. and Dascilloidea longitudinal epi- and hypopharyngeal bulges replaced by complex of adjacent and densely microtrichious (inframandibular) antepipharynx and prelabium, probably in correlation with extraoral digestion and intake of liquid food (Anton & Beutel, 2012).

81. Salivary glands: (0) present; (1) absent. Absent in Coleoptera and Strepsiptera (Beutel *et al.*, 2011).

82. Salivarium: (0) present; (1) absent. Prelabium and hypopharynx fused in Coleoptera and absent in Strepsiptera as defined separate elements of the head, salivarium thus absent in both groups (Anton & Beutel, 2004, 2012; Beutel & Pohl 2005; Lawrence *et al.*, 2011). Salivarium generally well developed in adults of Neuropterida (Achtelig, 1969; Aspöck & Aspöck, 2003, Ferris & Pennebaker, 1939; Beutel *et al.*, 2010b; Randolf *et al.*, 2013, 2014; Zimmermann *et al.*, 2011).

83. Mouthfield sclerite: (0) absent; (1) present. Present in Strepsiptera (Kinzelbach; 1971; Pohl & Beutel, 2005).

84. Balloon-gut: (0) absent; (1) present. Large part of digestive tract transformed into air-filled balloon gut in extant Strepsiptera (Beutel & Pohl, 2005; Pohl & Beutel, 2005).

Adults, prothorax

85. Cervical sclerites: (0) present; (1) absent; (2) vestigial. Present in Neuropterida (Ferris & Pennebaker, 1939; Czihak, 1953, 1957; Matsuda, 1956, 1970). Usually absent in Archostemata (Baehr, 1975) (vestigial in *Tetraphalerus* Waterhouse; Friedrich *et al.*, 2008). Always absent in Adephaga (Larsén, 1966; Baehr, 1979) and Myxophaga. Missing in some groups of Polyphaga (e.g. Tenebrionidae, Curculionidae). Distinctly reduced in Chrysomelidae and related families (Larsén, 1966).

86. Lateral connection of pronotum and propleuron: (0) absent; (1) partly or completely connected. Firmly connected in Coleoptera (e.g. Baehr, 1975; Friedrich *et al.*, 2008) and fused in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

87. Protibial apex: (0) without antenna cleaning organ; (1) antenna cleaning organ present. Present in Geadephaga (e.g. Beutel, 1992).

88. Prothoracic trochantin: (0) distinct sclerite; (1) fused with propleura; (2) fused with notum, sternum and pleura; (3) absent. Fused with propleura in Myxophaga and Polyphaga (Hlavac, 1972, 1975). Notum, sternum, pleura, and trochantin completely fused in *Micromalthus* (Lawrence & Newton, 1982). Absent in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

89. Propleura: (0) part of external body wall; (1) concealed, distinctly reduced in size; (2) fused with all other external sclerites of prothorax. Greatly reduced in size and internalized as cryptopleura in Polyphaga (Hlavac, 1972, 1975; Lawrence, 1982). Fused with other external prothoracic sclerites in *Micromalthus* (Lawrence & Newton, 1982) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012)

90. Spinasternum I: (0) well developed; (1) vestigial or absent. Present in Neuropterida (excl. Sialidae; Matsuda, 1970) and Cupedidae (Baehr, 1975; Beutel & Haas, 2000). Absent or strongly reduced in non-archostematan beetles (Doyen, 1966; Baehr, 1975; Friedrich *et al.*, 2008).

91. Apical part of procoxa: (0) without condyle; (1) condyle present. Ventral procoxal condyle present in Adephaga (excl. Gyrininae) (Baehr, 1979; Beutel, 1989b, 1997). Partly reduced in Dytiscidae (coded as 1) (Baehr, 1979).

92. Prothoracic defensive glands: (0) absent; (1) present. Present in Hygrobiidae and Dytiscidae (e.g. Beutel *et al.*, 2006).

93. M. pleuro-occipitalis (M. 7, Itpm1): (0) present; (1) absent. Present in *Chrysopa* (Miller, 1933), *Sialis* (Czihak, 1953), and *Corydalus* Latreille (Kelsey, 1954). Absent in *Agulla* Navás (Matsuda, 1956), *Myrmeleon* (Korn, 1943), and Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2008).

94. M. cervicale-occipitalis torquatus (M. 8, Idvm1?): (0) present; (1) absent. Absent in *Chrysopa* (Miller, 1933), Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2008) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

95. M. mesonoto-postpleuralis (M. 19, IItpm12?): (0) present; (1) absent. Absent in Neuroptera (Miller, 1933; Korn, 1943), Coleoptera (e.g. Larsén, 1966; Baehr, 1975; Friedrich *et al.*, 2008) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

96. M. prospina-mesopleuralis (M. 20, Ispm2): (0) present; (1) absent. Present in Raphidioptera (Matsuda, 1956; Friedrich & Beutel, 2010), Sialidae (Czihak, 1953), and Archostemata (Baehr, 1975; Friedrich *et al.*, 2008). Absent in Neuroptera (Korn, 1943), Corydalidae (Maki, 1936; Kelsey, 1957), non-archostematan Coleoptera (see Friedrich *et al.*, 2008; Friedrich & Beutel, 2010), Strepsiptera, and some other groups including Hymenoptera (Friedrich & Beutel, 2010).

97. M. profurca-spinalis (M. 21, Ivlm4): (0) present; (1) absent. Present in Raphidioptera (Matsuda, 1956; Friedrich & Beutel, 2010), Megaloptera (except Chauliodinae; Czihak, 1953; Kelsey, 1957), and many other groups (Friedrich & Beutel, 2010). Absent in Neuroptera (Korn, 1943; Friedrich & Beutel, 2010), Coleoptera (Baehr, 1975; Friedrich *et al.*, 2008) and Strepsiptera (Koeth *et al.*, 2012).

98. M. pronoto-coxalis posterior (M. 23, Idvm17): (0) present; (1) absent. Usually present in insects but generally missing in Coleoptera (Friedrich *et al.*, 2008). Whether the pronoto-coxal muscle of Strepsiptera is a homologous muscle is uncertain (Koeth *et al.*, 2012) (coded as ?).

99. M. procoxa-cervicalis (M. 26, Ipcm1/2): (0) present; (1) absent. A muscle connecting the procoxa and lateral cervical sclerite is present in Megaloptera (Maki, 1936; Czihak, 1953; Kelsey, 1954), in some groups of Neuroptera (e.g. Osmylidae,

Nevrorthidae), and in most other groups of insects. Absent in the myrmeleontiform lineage of Neuroptera (Korn, 1943; Czihak, 1957), in Raphidioptera (Matsuda, 1956), Coleoptera (Friedrich *et al.*, 2008), Strepsiptera (Koeth *et al.*, 2012), and some other groups (Friedrich & Beutel, 2010).

100. M. sterno-coxalis (M. 27, Iscm1): (0) present; (1) absent. Absent in *Myrmeleon* (Korn, 1943), Gyrininae (Larsén, 1966; Beutel, 1989b), Haliplidae, and most groups of Polyphaga (Larsén, 1966; Baehr, 1979; Beutel & Komarek, 2004; Ge *et al.*, 2007).

101. M. pleura-trochanteralis (M. 31, Ipcm8): (0) present; (1) absent. In contrast to Beutel & Haas (2000) present in Megaloptera (Czihak, 1953; Kelsey, 1954) and Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2008). Absent in *Agulla* (Raphidioptera; Matsuda, 1956), Neuroptera (Matsuda, 1970) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

102. M. prospina-mesofurcalis (Ivlm9): (0) present; (1) absent. Present in Neuropterida (e.g. Friedrich & Beutel, 2010), Cupedidae (Baehr, 1975), and many other groups of insects (Friedrich & Beutel, 2008, 2010). Missing in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012), non-archostematan Coleoptera (Larsén, 1966), and in some other groups of insects (e.g. Antliophora) (Friedrich & Beutel, 2010).

103. M. profurca-coxalis medialis (Iscm3): (0) present; (1) absent. Present in Neuroptera, Raphidioptera, Sialidae (Korn, 1943; Matsuda, 1956; Friedrich & Beutel, 2010), and most other groups of insects (Friedrich & Beutel, 2008, 2010). Absent in Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2008), Strepsiptera (Koeth *et al.*, 2012) and some other goups of insects (e.g. Trichoptera; Friedrich & Beutel, 2010).

104. M. prospina-coxalis (Iscm5): (0) present; (1) absent. Present in Neuroptera, Sialidae (Czihak, 1953) and many other groups of insects (e.g. Friedrich & Beutel, 2008, 2010). Absent in Raphidioptera (Matsuda, 1956), Corydalidae (Maki, 1936; Kelsey, 1957), Coleoptera (Larsén, 1966; Baehr, 1975; Friedrich *et al.*, 2008), Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012), and Antliophora (Friedrich & Beutel, 2010).

Adults, pterothorax

105. Relative size of pterothoracic segments: (0) almost equally sized; (1) mesothorax distinctly reduced in size. Almost equally sized in most groups of Neuropterida and Mecoptera (e.g. Maki, 1936; Korn, 1943; Czihak, 1953; Matsuda, 1956; Kelsey, 1957; Friedrich & Beutel, 2010). Metathorax strongly enlarged in Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2008) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

106. Relative size of wings: (0) equally sized; (1) hind wings distinctly larger. Modified fore wings of Coleoptera and Strepsiptera smaller than hind wings. Hind wings create main or exclusive propulsive force in flight (Kinzelbach, 1971; Friedrich *et al.*, 2008; Koeth *et al.*, 2012). Both pairs of nearly equal size in Neuropterida (e.g. Beutel *et al.*, 2011).

107. Wings of females: (0) present; (1) absent. At least forewings preserved as elytra in females of Coleoptera (with very few exceptions), and usually also membranous hind wings. Both pairs of wings absent in females of Strepsiptera (Kinzelbach, 1971; Pohl & Beutel, 2005).

108. Connection of meso- and metaventrites: (0) separated; (1) process of metaventrite articulates with mesoventrite; (2) both firmly connected. Distinctly separated from each other in Neuropterida, Strepsiptera and Archostemata (Baehr, 1975; Beutel & Haas, 2000). Metasternal process articulating with posterior mesoventrite in Adephaga (e.g. Beutel, 1992; Beutel & Haas, 2000). Rigidly connected to each other between and within mesocoxal cavities in Myxophaga and Polyphaga (Beutel & Haas, 2000) with few exceptions (Scirtoidea, Derodontidae, Leiodidae partim; e.g. Friedrich & Beutel, 2006; Ge *et al.*, 2007).

109. Katepisternal mesocoxal joint: (0) present; (1) absent. Almost generally present in non-coleopteran holometabolous groups (e.g. Friedrich & Beutel, 2010) and also in Ommatidae and Cupedidae (Baehr, 1975; Friedrich *et al.*, 2008). Absent in Adephaga, Myxophaga, and Polyphaga (Larsén, 1966; Beutel & Haas, 2000).

110. Mesothoracic transverse ridge: (0) present; (1) absent. Present in Neuropterida (e.g. Friedrich & Beutel, 2010), Cupedidae (Baehr, 1975), Ommatidae (Friedrich *et al.*, 2008) and *Sikhotealinia* Lafer (Jurodidae) (Beutel *et al.*, 2008). Absent in Micromalthidae, Crowsoniellidae and non-archostematan Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2008).

111. Origin of mesofurca: (0) between mesocoxae with common stem; (1) mesofurcal arms separated at base. Origin with common base between mesocoxae in Neuropterida (e.g. Friedrich & Beutel, 2010), Cupedidae and Ommatidae (Baehr, 1975; Friedrich *et al.*, 2008). Base of arms separated in *Micromalthus*, Adephaga (except for some Gyrinidae), Myxophaga, and Polyphaga (e.g. Larsén, 1966; Friedrich & Beutel, 2006).

112. Mesothoracic meron: (0) present; (1) absent (Larsén, 1945a). Meron and associated muscles absent in Coleoptera and Strepsiptera (Larsén, 1945; Friedrich & Beutel, 2006; Friedrich *et al.*, 2008; Koeth *et al.*, 2012).

113. Fore wings: (0) unsclerotized; (1) partly sclerotized, reticulate pattern with window punctures; (2) fully sclerotized; (2) transformed into halteres. Transformed into halteres in Strepsiptera (e.g. Kinzelbach, 1971) and into elytra with epipleura in Coleoptera (Lawrence & Newton, 1982). Reticulate pattern with window punctures present in stem-group Coleoptera, Cupedidae and Ommatidae (e.g. Beutel *et al.*, 2008).

114. Scutellar elytra-locking device: (0) absent; (1) present. Scutellar shield forms triangular elytra-locking device in Coleoptera (Heberdey, 1938).

115. Proximal part of mesocoxae: (0) not recessed into coxal cavities; (1) recessed into cavities. Recessed into cavities in Coleoptera (e.g. Lawrence, 1982).

116. M. mesoscutello-postnotalis (M. 41, IIdlm3): (0) present; (1) absent. Present in Neuropterida and other groups (e.g. Friedrich & Beutel, 2010). Absent in Coleoptera

(e.g. Larsén, 1966; Friedrich & Beutel, 2006, 2010), Strepsiptera (Koeth *et al.*, 2012) and most groups of Antliophora (Friedrich & Beutel, 2010).

117. M. mesosterni secundus (M. 43, IIvlm9): (0) present; (1) absent. Absent in *Micromalthus*, in some species of Adephaga, and in Polyphaga with the exception of *Lytta* Fabricius and *Meloe* Linnaeus (Meloidae) (e.g. Larsén, 1966; Beutel & Haas, 2000). Not clearly identified in *Tetraphalerus* (Friedrich *et al.*, 2008). Absent in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

118. Mm. mesonoto-sternales (M. 44, IIdvm1): (0) present; (1) absent. Absent in non-archostematan Coleoptera (e.g. Larsén, 1966; Baehr, 1975; Beutel & Komarek, 2004; Beutel & Haas, 2000). Present in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

119. M. mesonoto-pleuralis posterior (M. 46, IItpm6): (0) present; (1) absent. Present in Megaloptera (e.g. Maki, 1936; Czihak, 1953; Kelsey, 1957), Raphidioptera (Matsuda, 1956; Friedrich & Beutel, 2010), Archostemata (Baehr, 1975; Friedrich *et al.*, 2008), and some groups of Neuroptera (e.g. *Nevrorthus*, *Sisyra* Burmeister; Mickoleit, 1969; Friedrich & Beutel, 2010). Absent in non-archostematan Coleoptera (Larsén, 1966; Beutel & Haas, 2000), Strepsiptera (Koeth *et al.*, 2012), and some other groups including Hymenoptera (Friedrich & Beutel, 2010).

120. M. mesonoto-basalaris (M. 48, IItpm3): (0) present; (1) absent. Present in Neuropterida and most other groups of Holometabola, but absent in Coleoptera (e.g. Larsén, 1966; Friedrich & Beutel, 2006, 2010) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

121. M. mesanepisterno-sternalis (M. 50, IIspm1): (0) present; (1) absent. Present in Neuropterida and almost all other groups of Holometabola (Friedrich & Beutel, 2010). Absent in non-archostematan beetles (e.g. Larsén, 1966; Friedrich *et al.*, 2008) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

122. Mm. mesopleura-alares a and b (M. 53, M. 54, IItpm7, 9): (0) clearly separated; (1) single muscle or two branches inserting on one tendon. With separate origins and insertions in Neuropterida (Maki, 1936; Korn, 1943; Czihak, 1953; Kelsey, 1957), Cupedidae (Baehr, 1975), Ommatidae and *Micromalthus*, but not in non-archostematan Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2008). Muscles absent in Strepsiptera (Koeth *et al.*, 2012).

123. M. intramesanepisternalis (M. 56, IIppm1): (0) present; (1) absent. Absent in *Myrmeleon* (Neuroptera; Korn, 1943), Raphidioptera (Matsuda, 1956), Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2008) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

124. M. mesopleuracosto-praenotalis (M. 57, IItpm2): (0) present; (1) absent. Absent in Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2008) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

125. M. mesonoto-trochantinalis (M. 59, IIdvm2): (0) present; (1) absent. Absent in *Micromalthus* (mesotrochantin not exposed) and *Tetraphalerus* (Friedrich *et al.*,

2008), in some genera of Adephaga, in Myxophaga and Polyphaga (e.g. Larsén, 1966; Friedrich *et al.*, 2008), and in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

126. M. mesocoxa-subalaris (M. 64, IIdvm6): (0) present; (1) absent. Absent in Polyphaga (e.g. Larsén, 1966; Beutel & Haas, 2000). Present in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

127. M. mesanepisterno-trochantinalis (M. 68, IIpcm1): (0) present; (1) absent. Absent in *Myrmeleon* (Korn, 1943), Coleoptera (e.g. Larsén, 1966; Baehr, 1975; Friedrich *et al.*, 2008) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

128. M. mesonoto-trochanteralis (M. 69, IIdvm7): (0) present; (1) absent. Absent in *Sphaerius* and all polyphagans examined (e.g. Larsén, 1966; Friedrich & Beutel, 2006) and also in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

129. M. prophragma-mesanepisternalis (IItpm1): (0) present; (1) absent. Present in Neuropterida and other groups (Friedrich & Beutel, 2010). Missing in Coleoptera (Larsén, 1966; Friedrich *et al.*, 2008), Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012) and some other taxa, especially in wingless insects.

130. M. mesofurca-metanepisternalis (M. 88, IIspm6): (0) present; (1) absent. Present some holometabolan groups including Hymenoptera, Neuroptera (e.g. *Nevrorthus*, Osmylidae), Raphidioptera, Sialidae (Friedrich & Beutel, 2010), and Archostemata (Baehr, 1975; Friedrich *et al.*, 2008). Absent in non-archostematan beetles, in Strepsiptera, and in Antliophora (Friedrich & Beutel, 2010).

131. M. mesonoto-pleuralis medialis (IItpm5): (0) present; (1) absent. Present in Megaloptera (Maki, 1936; Czihak, 1953; Kelsey, 1957), Raphidioptera (Matsuda, 1956), some groups of Neuroptera (Nevrorthidae, Osmylidae), and most other groups of Holometabola (Friedrich & Beutel, 2010). Always absent in Coleoptera (Friedrich *et al.*, 2008) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012), and also in Siphonaptera and other wingless insects (Friedrich & Beutel, 2010).

132. Mesal metacoxal walls: (0) not fused; (1) metacoxae attached to each other along ventromedian edge; (2) mesal walls fused. Fused in adults of Trachypachidae and Dytiscoidea (e.g. Beutel & Roughley, 1987, 1988). Connected along ventromesal edges in Gyrininae.

133. Transverse metascutal fissure: (0) absent; (1) present (Brodsky, 1994). Membranous, transverse metascutal fissure (or area) present in Coleoptera (Campau, 1940; Doyen, 1966; Larsén, 1966; Brodsky, 1994; Ge *et al.*, 2007 ; Friedrich *et al.*, 2008).

134. Metathoracic elytra-locking device: (0) absent; (1) present. Alacristae present and forming locking device in Coleoptera (e.g. Heberdey, 1938; Friedrich *et al.*, 2008).

135. Median division of metapostnotum: (0) absent; (1) present. Present in Neuropterida (Achtelig, 1975).

136. Metacoxae: (0) transverse, recessed into cavities; (1) not transverse, not recessed into cavities. Distinctly transverse in Coleoptera with few exceptions (e.g. Larsén, 1966; Friedrich *et al.*, 2008).

137. Metathoracic trochantin: (0) broad, well developed; (1) reduced, not visible externally; (2) absent. Well developed and visible externally in Neuropterida (Friedrich & Beutel, 2010), Cupedidae and Ommatidae (Baehr, 1975; Friedrich *et al.*, 2008). Absent from external surface in non-archostematan beetles (e.g. Friedrich & Beutel, 2006; Friedrich *et al.*, 2008). Absent in Strepsiptera (Koeth *et al.*, 2012).

138. Number of costal cross veins: (0) less than five; (1) more than five. More than five in Neuropterida (e.g. Beutel *et al.*, 2011).

139. Hind wing folding: (0) absent; (1) longitudinal and transverse hind wing folding, wings completely covered under elytra in repose. Folded in Coleoptera with very few exceptions (Haas, 1998; Beutel & Haas, 2000; Haas & Beutel, 2001).

140. Apical part of hind wing in resting position: (0) unfolded; (1) rolled; (2) folded. Apical part rolled in Archostemata, *Spanglerogyrus*, Haliplidae, in some small dytiscids, and in few representatives of Polyphaga (e.g. *Artematopus* Perty; Kukalová-Peck & Lawrence, 1993). Folded in other adults of Coleoptera examined (Beutel & Haas, 2000).

141. Oblongum of hind wing: (0) present; (1) absent. Present in Ommatidae, Cupedidae, Myxophaga (with few exceptions), and Adephaga (Beutel & Haas, 2000; Beutel *et al.*, 2008).

142. Subcubital binding patch: (0) absent; (1) present. Present in *Trachypachus* Motschulsky and Dytiscoidea (excl. Hygrobiidae; Beutel & Roughley, 1988; Beutel & Haas, 2000), and also in some groups of Polyphaga (Heberdey, 1938).

143. Anterior margin of hind wing: (0) not flexible; (1) flexible a bending zone; (2) with a hinge; (3) with a marginal joint. Anterior margin flexible in Coleoptera, but not in outgroup taxa (Haas, 1998; Haas & Beutel, 2001). With a bending zone in almost all groups Polyphaga but with a hinge in the remaining suborders (Kukalová-Peck & Lawrence, 1993, 2004). A marginal joint is present in Scarabaeoidea (Kukalová-Peck & Lawrence, 1993, 2004).

144. Bending zone in medial bar of hind wing: (0) present; (1) absent. Present in Archostemata, Adephaga and Myxophaga (Kukalová-Peck & Lawrence, 1993). Absent in Polyphaga (Kukalová-Peck & Lawrence, 1993) except for Scirtidae and Eucinetidae (Friedrich & Beutel, 2006).

145. Transverse veins of hind wing: (0) present; (1) absent. Generally absent in Strepsiptera with the exception of a single transverse vein in †*Mengea* Grote (Pohl & Beutel, 2005).

146. Shape of wing: (0) elongate, not extended rostrocaudally; (1) fan-shaped, rostrocaudally extended. Fan shaped in Strepsiptera excl. †Protoxenidae, with a rostrocaudally extended posterior region (Kinzelbach, 1971; Pohl & Beutel, 2005).

147. Distal part of MP_{1+2}: (0) straight or bent anteriorly; (1) bent posteriorly. MP_{1+2} straight or bent anteriorly in non-coleopteran Endopterygota, Archostemata, Adephaga and Hydroscaphidae. Bent posteriorly in all adults of Polyphaga examined (Haas, 1998; Friedrich & Beutel, 2006).

148. Triangular fold: (0) completely absent; (1) RA_{3+4} cut twice by triangular fold; (2) RA_{3+4} not cut twice by triangular fold. Basal portion of RA_{3+4} cut twice by triangular fold in Archostemata, Myxophaga, and Adephaga, but not in Polyphaga (Kukalová-Peck & Lawrence, 1993, 2004).

149. Fulcrum: (0) underneath 2nd axillary; (1) underneath 1st and 2nd axillary; (2) underneath 1st axillary. Fulcrum placed underneath 2nd axillary in Archostemata and under 1st axillary in Myrmeleontidae, Adephaga, Myxophaga, and Polyphaga. Located under both axillary sclerites in Megaloptera and Raphidioptera (Hörnschemeyer, 1998; Friedrich & Beutel, 2006).

150. 2nd axillary: (0) without a lateral process; (1) process present. Lateral process originating from ventral side of 2nd axillary present in non-archostematan beetles. Absent in Neuropterida and Archostemata (Hörnschemeyer, 1998).

151. Angle between the axis anterior notal process-1st axillary and the disto-cranial margin of 1st axillary: (0) 50° or more; (1) less than 45°. Angle of 50° or more in Neuropterida, Archostemata, Adephaga and Myxophaga (Hörnschemeyer, 1998). In Polyphaga 45° or less (Hörnschemeyer, 1998; Friedrich & Beutel, 2006).

152. Shape of metapostnotum: (0) not enlarged and plate-like or shield-like; (1) enlarged and plate-like or shield-like. Enlarged and plate-like in †Protoxenidae (Pohl *et al.*, 2005), very large and shield like in all other strepsipterans (Pohl & Beutel, 2005).

153. M. metascutello-postnotalis (M. 81, IIIdlm3): (0) absent; (1) present. Present in most groups of Holometabola including Neuropterida (Czihak, 1953, 1957; Matsuda, 1956; Kelsey, 1957; Friedrich & Beutel, 2010). Absent in Coleoptera (e.g. Baehr, 1975; Friedrich *et al.*, 2008), Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012) and Antliophora (Friedrich & Beutel, 2010).

154. M. metasterni primus (M. 82, IIvlm3): (0) present; (1) absent. Present in Coleoptera except for *Hydroscapha*, *Sphaerius* and very few polyphagans (e.g. *Cantharis* Linnaeus; Larsén, 1966; Friedrich *et al.*, 2008) and also in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

155. M. metasterni secundus (M. 83, IIvlm5): (0) present; (1) absent. Present in most outgroup taxa (Matsuda, 1970), in Cupedidae (Baehr, 1975), Ommatidae (Friedrich *et al.*, 2008), Trachypachidae (Beutel, 1988), in *Haliplus* Latreille, and in most groups of Carabidae (Larsén, 1966). Absent in Neuroptera (Korn, 1943; Czihak, 1957), Myxophaga, Polyphaga (Friedrich & Beutel, 2006), and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

156. M. metanoto-episternalis brevis (M. 89, IIItpm1): (0) present; (1) absent. Present in Neuroptera (Korn, 1943; Matsuda, 1970), Megaloptera (Maki, 1936; Czihak, 1953; Kelsey, 1957) and Archostemata (Baehr, 1975; Friedrich *et al.*, 2008). Absent in all other groups of Coleoptera (e.g. Larsén, 1966; Beutel & Haas, 2000) and also missing in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

157. M. metanoto-pleuralis medialis (M.91, IIItpm5): (0) present; (1) absent. Present in Neuropterida (except Osmylidae; Czihak, 1953; Matsuda, 1956) and

several other holometabolan groups including Hymenoptera (e.g. Friedrich *et al.,* 2010). Always absent in Coleoptera (see Friedrich *et al.,* 2009) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012), and also missing in wingless groups (Friedrich & Beutel, 2008, 2010).

158. M. metanoto-pleuralis b (M. 92, IIItpm6): (0) present; (1) absent. Present in Neuropterida (Maki, 1936; Korn, 1943; Czihak, 1953; Kelsey, 1957; Matsuda, 1970) and Cupedidae (baehr, 1975; in contrast to Beutel & Haas, 2000). Absent in *Tetraphalerus* and *Micromalthus*, in non-archostematan beetles (e.g. Larsén, 1966; Friedrich *et al.*, 2008), and in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

159. Mm. metapleura-alares a and b (Mm. 95, 96, IIItpm7, 9): (0) separate; (1) with common insertion (Larsén, 1966: M71). Areas of origin and insertion separated in Neuropterida (Maki, 1936; Korn, 1943; Czihak, 1953; Kelsey, 1957; Matsuda, 1970), Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012), and Archostemata (Baehr, 1975; Friedrich *et al.*, 2008). Common insertion on small sclerite proximad 3rd axillary in non-archostematan beetles (e.g. Larsén, 1966).

160. M. metafurca-pleuralis (M. 99, IIIspm2): present; (1) absent. Absent in Coleoptera (Larsén, 1966; Beutel & Haas, 2000). Present in Strepsiptera in contrast to Beutel & Haas (2000) (Koeth *et al.*, 2012).

161. M. metanoto-trochantinalis (M. 100, IIIdvm2): (0) present; (1) absent. Absent in *Tetraphalerus*, Adephaga, *Hydroscapha* LeConte, Sphaeriusidae, Hydrophilidae and *Cetonia* Fabricius (e.g. Larsén, 1966; Beutel & Haas, 2000; Friedrich *et al.*, 2008). Also missing in Strepsiptera (Koeth *et al.,* 2012).

162. M. metanoto-coxalis posterior (M. 102, IIIdvm5): (0) present; (1) absent. Absent in *Hydroscapha*, Sphaeriusidae and *Niptus* Boieldieu (Larsén, 1966; Beutel & Haas, 2000), and also in Strepsiptera (Kinzelbach, 1971; Koeth *et al.,* 2012).

163. M. metanepisterno-coxalis (M. 103, IIIpcm4): (0) present; (1) absent. Absent in Adephaga, Elateridae and Scirtoidea (Larsén, 1966; Friedrich & Beutel, 2006), and also missing in Strepsiptera (Kinzelbach, 1971; Koeth *et al.,* 2012).

164. M. metasterno-coxalis (M. 106, IIscm7?): (0) present; (1) absent. Absent in Coleoptera (except for *Ips* De Geer) (Larsén, 1966; Friedrich *et al.*, 2008) and also missing in Strepsiptera (Kinzelbach, 1971; Koeth *et al.,* 2012).

165. M. metafurca-coxalis posterior (M. 109, IIIscm2): (0) present; (1) absent. Present in Neuropterida (Friedrich & Beutel, 2010) and all beetles examined with the exception of Dytiscoidea (Larsén, 1966; Beutel & Haas, 2000) and Scirtoidea (Friedrich & Beutel, 2006). Absent in Strepsiptera (Koeth *et al.*, 2012).

166. M. metanepisterno-trochantinalis (M. 110, IIIpcm1): (0) present; (1) absent. Absent in Coleoptera (Larsén, 1966; Beutel & Haas, 2000; Friedrich *et al.*, 2008), Strepsiptera (Koeth *et al.*, 2012), and Neuroptera (Korn, 1943).

167. M. metatrochantero-basalaris (M. 112, IIIpcm5): (0) present; (1) absent. Present in Neuropterida (Friedrich & Beutel, 2010). Absent in all non-archostematan beetles and *Tetraphalerus* (Larsén, 1966; Friedrich *et al.*, 2008). Also absent in Strepsiptera (Koeth *et al.*, 2012).

168. Muscle between metafurcal arm and first abdominal stigma: (0) present; (1) absent. Present in Neuropterida but absent in Coloptera and Strepsiptera (Larsén, 1966; Friedrich *et al.*, 2008; Koeth *et al.*, 2012). Also missing in other holometabolous groups (Achtelig, 1975; Friedrich & Beutel, 2010).

Adults, abdomen

169. Transverse suture of abdominal tergite I: (0) absent; (1) present. Present in Neuropterida (Achtelig, 1975).

170. Abdominal sternite I: (0) strongly reduced or absent, not visible externally; (1) present, exposed. Reduced in Coleoptera (Lawrence, 1982; Beutel, 1997) (Fig. 2). Partly integrated into metathorax in Strepsiptera (Kinzelbach, 1971; coded as 0).

171. Transverse suture of abdominal sternite II: (0) absent; (1) present. Present in Neuropterida (Achtelig, 1975).

172. Division of abdominal sternite II: (0) not divided by hind coxae; (1) completely divided. Completely divided in Adephaga (Lawrence *et al.*, 2011).

173. Median ridge of sternite II: (0) absent; (1) present. Present in Cupedidae and Ommatidae (Beutel *et al.*, 2008).

174. Abdominal segments IX and X: (0) exposed; (1) retracted into abdominal apex. Concealed within the preceding abdominal segments in Coleoptera (e.g. Lawrence, 1982) (Fig. 2).

175. Configuration of segment IX: (0) not ring-shaped and strongly sclerotized; (1) ring-shaped and strongly sclerotized. Ring-shaped and strongly sclerotized in males of Strepsiptera (Pohl & Beutel, 2005, 2008).

176. Shape of male tergite X: (0) not narrowed and elongated; (1) narrowed and elongated. Narrow and elongated in males of Strepsiptera (Pohl & Beutel, 2005, 2008).

177. Genital appendages IX of females: (0) separate; (1) fused, with intrinsic muscles; (2) absent. Fused and equipped with intrinsic muscles in females of Neuropterida (Mickoleit, 1973). Absent in Strepsiptera (Kinzelbach, 1971; Pohl & Beutel, 2005, 2008).

178. Trichobothria field on tergum X: (0) absent; (1) present. Present in Neuropterida (Aspöck & Aspöck, 2008).

179. Arrangement of trichobothria field on tergum X: (0) band-shaped; (1) rosette-shaped. Rosette-shaped in Neuroptera and Megaloptera (Aspöck & Aspöck, 2008).

180. Eversible sacs of segment XI: (0) absent; (1) present. Present in both subgroups of Megaloptera, assigned to fused gonocoxites XI by Aspöck & Aspöck (2008).

181. Number of Malpighian tubules in adults: (0) eight; (1) six; (2) four; (3) vestigial or absent. Eight in Neuroptera (excl. Coniopterygidae) and, six in Raphidioptera, Sialinae (Aspöck & Aspöck, 2003), Myxophaga, Hydrophiloidea, Eucinetoidea, Derodontidae, and some other groups of Polyphaga (Lawrence, 1982). Four in Mengenillidae (females), Archostemata, Adephaga, and many groups of

Polyphaga (Lawrence, 1982). Usually reduced in Strepsiptera. Vestigial but recognizable in males *Xenos* (Kinzelbach, 1971; Pohl & Beutel, 2005, 2008).

182. Condition of Malpighian tubules in adults: (0) free; (1) cryptonephric. Cryptonephric in Cucujiformia (Lawrence, 1982; Lawrence *et al.*, 2011) and terrestrial larvae of Neuroptera (Aspöck & Aspöck, 2003; coded as 0 for neuropteran terminals). Coded as inapplicable for Strepsiptera.

Characters related to reproduction and development

183. Torsion of aedeagus: (0) absent; (1) present. Rotated during repose (90°) and during copulation (180°) in Adephaga (excl. Gyrinidae) (Beutel & Roughley, 1988). Also everted asymmetrically in Scydmaeninae and other subgroups of Staphylinidae (Beutel & Leschen, 2005; Lawrence *et al.*, 2011; Newton, 2016).

184. Configuration of aedeagus: (0) composed of different elements, not simple and blade like; (1) simple and blade-like. Aedeagus usually composed of basal piece, parameres and median lobe in beetles (Beutel, 1997; Lawrence, 1982). Also, structurally complex in Neuropterida (Aspöck & Aspöck, 2008). Simple and blade-like in Strepsiptera (Kinzelbach, 1971; Pohl & Beutel, 2005). Male genitalia highly variable in *Zorotypus* (coded as ?).

185. Ovarioles: (0) developed; (1) reduced. Completely reduced in females of Strepsiptera, eggs float in body cavity (Kinzelbach, 1971; Pohl & Beutel, 2005, 2008).

186. Birth organs: (0) absent; (1) present. Present in females of Strepsiptera (Kinzelbach, 1971; Pohl & Beutel, 2005, 2008).

187. Mode of copulation: (0) not traumatic; (1) traumatic. Traumatic insemination or penetration in Strepsiptera (Silvestri, 1949; Peinert *et al.*, 2016).

188. Mode of reproduction: (0) oviparous; (1) viviparous. Viviparous in Strepsiptera (Kinzelbach, 1971; Kathirithamby, 1989; Pohl & Beutel, 2005, 2008).

189. Egg deposition: (0) laid without cocoon or egg case; (1) one side of single egg covered by web; (2) eggs enclosed in silk cocoons or egg case. One side of single egg covered by web in Hydraenidae, eggs enclosed in silk cocoon or egg case in Hydrophiloidea (Hansen, 1991, 1997). Coded as inapplicable for Strepsiptera.

190. Puparium: (0) absent; (1) present. Formed by exuvia of last secondary larva in Strepsiptera (e.g. Kinzelbach, 1971; Pohl & Beutel, 2005, 2008) (Fig. 1D).

RESULTS

The parsimony analysis of the complete matrix (190 characters) with TNT yielded 12 minimum length trees (83 with NONA) of 402 steps length, with a pattern Strepsiptera + (Archostemata + (Adephaga + (Myxophaga + Polyphaga))) (Fig. 4: strict consensus tree). The monophyly of Coleopterida is very strongly supported (branch support (=bs): 15) whereas it is quite low for Coleoptera (bs: 2) and also for Coleoptera excl. Archostemata (bs: 2). The branch support is 6 for Adephaga, 3 for Myxophaga + Polyphaga, 4 for Myxophaga, and 5 for Polyphaga.

The Bayesian analysis (Figs A1, A2) yielded a similar pattern, but with the noteworthy exception that the strepsipteran terminals are placed as sister group of Coleoptera excl. Archostemata (posterior probability (=pp): 0.769). The posterior probability for Coleopterida and Strepsiptera was 1. In parsimony analyses a pattern (Polyphaga (+ Adephaga + (Myxophaga + Archostemata))) (25) required 9 additional steps (tree length 411) and (Polyphaga (+ Archostemata + (Adephaga + Myxophaga))) 11 (tree length 413) (25, 26).

Unambiguous apomorphies of major clades obtained in parsimony analysis (homoplasious changes in italics) (see Fig. 4)

Coleopterida (branch support (=bs): 17)
14.1. *M. frontolabralis absent in larvae* (present in 1st instars of *Tenomerga*); 34.2. Salivary ducts and glands absent; 64.2. Ocelli absent (with few reversals in Coleoptera); 65.1. M. frontolabralis absent; 81.1. Salivary glands absent; 82.1. Salivarium absent; 85.1. *Cervical sclerites absent* (vestigial in Ommatidae and present in many groups of Polyphaga); 86.1. Pronotum and propleuron laterally connected; 94.1. M. cervicale-occipitalis torquatus absent; 102.1. M. prospina-mesofurcalis absent (implies reversal in Archostemata); 103.1. M. profurca-coxalis medialis (Iscm 3) absent; 112.1. Mesocoxal meron absent; 116.1. M. mesoscutello-postnotalis (M. 41, IIdlm3) absent; 120.1. M. mesonoto-basalaris (M. 48, IItpm3) absent; 125.1. M. mesonoto-trochantinalis (M. 59, IIdvm2) absent; 129.1. M. prophragma-mesanepisternalis (IItpm1) absent; 161.1. M. metanoto-trochantinalis (M. 100, IIIdvm2) absent; 164.1. M. metasterno-coxalis (M. 106, IIscm7?) absent; 170.0. Abdominal sternite I strongly reduced or absent; 181.2. *Four Malpighian tubules* (?).

Strepsiptera (bs 48, pp 1)
1.1. Length of 1st instars less than 0.5 mm; 3.1. Tentorium of larvae reduced; 6.2. Head of endoparasitic secondary larvae rounded and simplified; 12.2. Larval labrum completely fused with head capsule; 13.1. Anterior margin of head forms sharp cutting edge; 15.1. M. frontoepipharyngalis absent; 16.1. Antenna of primary larva extremely reduced; 19.2. Mandibles largely internalized, ventral mouthparts partly fused with head capsule; 24.3. Maxillae medially connected, plate-like; 27.2. Galea absent in primary larvae; 29.1. Maxillary palp present as bolt-shaped socket and seta; 33.1. Double opening of preoral cavity; 35.1. Brain shifted to middle body region; 37.1. Sternal plates present; 39.2. Five leg segments, femur fused with trochanter; 40.2. Lobe-like pretarsal attachment pads; 42.1. Posterior edges of abdominal sternites with bristles and spinuale; 50.1. Terminal abdominal jumping apparatus of 1st instars present; 52.1. Larvae endoparasitic; 56.1. Cuticle of males with dense vestiture of microtrichia; 59.1. Frontal region with V- or U-shaped impression; 63.1. Raspberry compound eyes; 67.1. Antennal flabella present; 68.1. Hofeneder's organ present; 69.1. Dense vestiture of antennal dome-shaped chemoreceptors; 75.1. Maxillary palp

1-segmented; 77.1. Prementum fused with other labial parts and head capsule; 83.1. Mouthfield sclerite; 84.1. Balloon gut; 88.2. Protrochantin absent; 89.2. *Propleura fused with all other external prothoracic sclerites*; 107.1. Females completely wingless; 117.1. *M. mesosterni secundus (M. 43, Ilvlm9) absent*; 128.1. *M. noto-trochanteralis mesothoracis (M. 69, IIdvm7) absent*; 137.2. Metatrochantin reduced, not visible externally; 145.1. Transverse veins of hind wings absent; 146.1. Hind wings fan-shaped; 152.1. Metapostnotum enlarged, plate-like or shield-like; 162.1. *M. noto-coxalis posterior metathoracis (M. 102, IIIdvm5) absent*; 165.1. *M. furca-coxalis posterior metathoracis (M. 109, IIIscm2) absent*; 175.1. Segment IX ring-shaped and fully sclerotized; 176.1. Tergite X of males elongated and narrow; 177.2. Female genital appendages IX absent; 184.1. Aedeagus formed o single element and blade-like

Coleoptera (bs: 1)
53.1. Pupal mandible immobilized; 54.1. Sclerites firmly connected, no membranes exposed; 114.1. Scutellar elytra-locking device present; 115.1. Proximal part of mesocoxae recessed into cavities; 133.1. Transverse metascutal fissure present; 134.1. Alacristae forming metascutal elytral locking device; 139.1. Longitudinal and transverse hind wing folding; 141.0. Oblongum of hind wing present; 143.1. Anterior margin of hind wing with a hinge; 160.1. M. metafurca-pleuralis (M. 99, IIIspm2) absent; 174.1. Abdominal segments IX and X retracted into abdominal apex.

Coleoptera excluding Archostemata (bs: 1)
118.1. Mm. mesonoto-sternales (M. 44, IIdvm1) absent; 122.1. Mm. mesopleura-alares a and b (M. 53, M. 54, IItpm7, 9) present as single muscle or two branches inserting on one tendon; 137.1. Metatrochantin absent from surface; 150.1. Lateral process of 2nd axillary present; 159.1. Mm. metapleura-alares a and b (Mm. 95, 96, IIItpm7, 9) with common insertion.

Polyphaga + Myxophaga (bs: 3)
5.0. *Larval head subprognathous*; 17.3. Three larval antennomeres; 38.1. *Larval leg with single claw*; 39.1. *Larval leg with tibia and tarsus fused*; 72.1. *Mandible of adults with mola*; 80.1. *Dorsal and ventral wall of preoral cavity with longitudinal epi- and hypopharyngeal bulges with fields of microtrichia* (modified or absent in many groups of Polyphaga; 88.1. Protrochantin fused with propleura.

Polyphaga (bs: 4)
85.0. *Cervical sclerites well developed* (absent in several groups); 89.1. Propleura internalized; 100.1. *M. sterno-coxalis (M. 27, Iscm1) absent* (also absent in some adephagans); 117.1. *M. mesosterni secundus (M. 43, Ilvlm9) absent* (occurring in Meloidae); 126.1. M. mesocoxa-subalaris (M. 64, IIdvm6) absent; 141.1. *Oblongum of hind wing absent*; 147.1. Anterior margin of hind wing with a bending zone; 148.2.

Bending zone in medial bar of hind wing absent; 151.1. Angle between the axis anterior notal process and the disto-cranial margin of 1st axillary less than 45°.

For autapomorphies of Archostemata, Myxophaga and Adephaga see Beutel (1993, 1997), Beutel & Hörnschemeyer (2002a, b), Beutel *et al.* (1999, 2006), and Lawrence *et al.* (2011).

Discussion

The monophyly of Coleopterida

The morphological support for a clade comprising Strepsiptera and Coleoptera was considered weak or moderate in earlier studies (Friedrich *et al.*, 2010; Beutel *et al.*, 2011). A surprising result in the present contribution was a very strong branch support value for this unit (bs 17, pp: 1) in the parsimony analysis and numerous potential synapomorphies, only some of them linked with posteromotorism. Shared derived features of the adult head are the reduced number of antennomeres, the loss of M. frontolabralis, and the absence of the salivarium, salivary ducts and salivary glands. Another potential synapomorphy is the far-reaching or complete reduction of the ocelli, which are always absent in strepsipterans and present as small vestiges in a few groups of Coleoptera, arguably due to reversal (Leschen & Beutel, 2004; Beutel *et al.*, 2008). Derived features of the prothorax are the lateral connection of the pronotum and propleuron and the loss of four muscles, two of them associated with the prospina and one belonging to the neck region. The loss of the mesocoxal meron and the separation of the mesofurcal arms are derived features of the mesothorax. Several muscle losses are likely linked to posteromotorism (not coded as a single character here), with a distinctly shortened mesothorax with halteres or elytra, respectively, and an enlarged metathorax with the functional wings (Figs 1-3). This clearly distinguishes Coleopterida from all other groups of Holometabola, which are either characterized by similarly sized pterothoracic segments and wing pairs (Neuropterida, Mecoptera) or by anteromotorism, either with functional (Hymenoptera) or anatomical (Diptera) dipterism (Beutel *et al.*, 2011; Peters *et al.*, 2014). Characters of the abdomen play a minor role if at all. The reduced first abdominal sternite is apparently linked with the enlargement of the metathorax and metacoxae. The reduced number of four Malpighian tubules is likely due to convergence, as six are present in many beetles (Lawrence, 1982). Even though the minute first instars of Strepsiptera appear quite similar to larvae of some groups of Coleoptera, notably in the predacious Adephaga, our analyses did not yield any synapomorphies of the immature stages. A large and distinctly sclerotized pronotum is arguably a shared derived feature, even though this varies strongly in Coleoptera and more or less distinctly defined pronota also occur in other groups, especially in Neuropterida (Beutel *et al.*, 2010a). Prognathism and the fused labrum are features linked with carnivorous habits in some groups of Coleoptera (e.g. Adephaga, Hydrophiloidea,

Elateroidea) (Beutel, 1993, 1995, 1999), but with endoparasitism in the case of Strepsiptera (Kinzelbach, 1971; Pohl & Beutel, 2008, 2013), i.e. with the necessity to penetrate the host's body wall.

A surprising result of the Bayesian analysis of the morphological data was the inclusion of Strepsiptera in Coleoptera, thus rendering the latter paraphyletic (Figs A1, A2). The placement of the strepsipteran terminal as sister group of Myxophaga + Polyphaga is in contrast to earlier hypotheses with 'Stylopidae' as a specialized family of cucujiform Polyphaga (7), and also to the recent evaluations of genomes (Niehuis *et al.*, 2012) and transcriptomes (Peters *et al.*, 2014; Misof *et al.*, 2014; Boussau *et al.*, 2014) supporting Coleoptera as a clade. Numerous autapomorphies of Strepsiptera suggest long branch attraction (LBA) as a possible reason for the apparent artifact. However, likelihood analyses (including Bayesian inference) are considered less sensitive to LBA than parsimony (Bergsten, 2005; Kjer *et al.*, 2016). Moreover, Strepsiptera were even more deeply nested in Coleoptera (sister to Myxophaga + Polyphaga) after the exclusion of 36 presumptive autapomorphies of Strepsiptera. It is conceivable that establishing a suitable model for the data is a problem in Bayesian analyses of morphological characters. On principle, it cannot be fully excluded that Strepsiptera are nested in Coleoptera (McKenna & Fareell, 2009). However, this would not only be in clear contrast to recent analyses of genomic and transcriptomic data (Niehuis *et al.*, 2012; Peters *et al.*, 2014; Misof *et al.*, 2014; Boussau *et al.*, 2014), but also imply unlikely transformations of morphological characters, such as the formation of gyroscopic sense organs from elytra, extensive secondarily exposed membranes, and secondarily everted and very specifically modified terminal abdominal segments.

Numerous apomorphies of Strepsiptera are distributed among all life stages and both sexes (e.g. Pohl & Beutel, 2005, 2008) (Fig.1). Some of them are linked with the exceptional flight performance of the adult males (Fig. 1G, H), for instance the dense vestiture of microtrichia on the body surface or the unusually large dorsal longitudinal muscles in the metathorax (Fig. 3). However, most of them are more or less closely related with endoparasitism, as for instance, the attachment structures and reduced antennae of the minute primary larvae, the far-reaching simplification of the secondary larvae (not coded here as single character) (Fig. 1A-D), the birth organs and reduced ovaries of females, vivipary and traumatic insemination or penetration (Silvestri, 1941; Peinert *et al.*, 2016), and the raspberry compound eyes (2, 107, 108) and sperm pump of the short-lived males (Kinzelbach, 1971; Pohl & Beutel, 2005, 2008).

It is noteworthy that the inclusion of strepsipteran terminal taxa in this study (in contrast to Beutel & Haas (2000) and Friedrich *et al.* (2009)) and additional character sets (mainly from Friedrich & Beutel (2010) and Beutel *et al.* (2011)) resulted in distinctly reduced branch support for Coleoptera (Fig. 4), 1 compared to more than 20 in Beutel & Haaas (2000) and Friedrich *et al.* (2009). Features previously interpreted as groundplan apomorphies of beetles have likely evolved earlier in the stem group of

Coleopterida. This includes posteromotorism as the most conspicuous character complex but also features of other body parts as outlined above. Despite the comparatively low branch support value, Coleoptera are still clearly supported by 10 autapomorphic character states, especially features frequently associated with a strongly armored body including the absence of external membranes, the formation of elytra, and invaginated terminal segments.

The coleopteran subordinal relationships

The phylogeny presented here (Fig. 4) is compatible with the results of earlier cladistic studies based on morphological data (Beutel & Haas, 2000; Beutel *et al.*, 2008; Friedrich *et al.*, 2009). Archostemata are placed as sister group of the remaining three suborders, and Myxophaga as sister group of Polyphaga (also in Bayesian analyses with Coleoptera enforced as monophyletic: Fig. A3). It is noteworthy that the present extended data set, as in the case of the entire Coleoptera, reduces the support value of this possible clade compared to previous analyses (Beutel & Haas, 2000; Friedrich *et al.*, 2009), 1 versus 5 or 11, respectively. The potential synapomorphies of Coleoptera excluding Archostemata were already discussed in earlier studies (Beutel & Haas, 2000; Beutel *et al.*, 2008; Friedrich *et al.*, 2009). That Archostemata (especially Cupedidae and Ommatidae) are the coleopteran subgroup with a maximum of preserved plesiomorphies is largely undisputed (Lawrence, 1999; Beutel & Haas, 2000). This includes the cuticular pattern with tubercles and scales, which is very similar to the cuticular surface of Permian stem group coleopterans (e.g. †Tshekardocoleidae, †Permocupedidae; (Beutel *et al.*, 2008; Ponomarenko, 1969), elytra with rows of unsclerotized window punctures, the presence of a transverse ridge on the mesoventrite, loosely connected meso- and metaventrites, an exposed metatrochantin, and a pterothoracic muscle set distinctly more complex than in the other suborders, especially Myxophaga and Polyphaga (Beutel & Haas, 2000).

Similar to previous analyses based on morphology, but in contrast to Kukalová-Peck & Lawrence (1993, 2004) and molecular studies (e.g. Peters *et al.*, 2014; Misof *et al.*, 2014; McKenna *et al.*, 2015), the small order Myxophaga is placed as sister group of the megadiverse Polyphaga. This is suggested by several shared derived features of larvae and adults, in the former the fusion of the tibia and tarsus and a single claw, and in the latter the fusion of the protrochantin and propleura (Hlavac, 1972, 1975; Lawrence, 1982; Beutel, 1997; Beutel & Haas, 2000). Additionally, the meso- and metaventrite are firmly connected in all myxophagans and almost all groups of Polyphaga, with the notable exception of Scirtoidea (and a few members of Leiodidae) (e.g. Beutel & Haas, 2000; Friedrich & Beutel, 2006; Ge *et al.*, 2007).

An important character and potential synapomorphy of Polyphaga and Myxophaga is a complex feeding apparatus with epi- and hypopharyngeal bulges with fields of microtrichia interacting with mandibular brushes (Anton & Beutel, 2004, 2012; Anton *et al.*, 2016; Antunes-Carvalho *et al.*, 2017). This suggests feeding on

decaying materials (saprophagy) or small particles (e.g. fungal spores) as a groundplan feature of both suborders. It is conceivable that this preoral configuration was secondarily lost in the other two suborders, in the case of Adephaga in correlation with carnivorous habits and preoral digestion, and in the case of Archostemata linked with limited food uptake of the adults. In this case, the presence of the preoral apparatus would be a complex derived groundplan feature of Coleoptera.

An alternative phylogenetic scenario is a placement of Polyphaga as sister group of the remaining three suborders. This was suggested based on characters of the hind wing (Kukalová-Peck & Lawrence, 1993, 2004), in recent molecular studies based either on transcriptomes (Peters *et al.*, 2014; Misof *et al.*, 2014) or analyses of 8 single genes (Wiegmann *et al.*, 2009), and also by unpublished results of analyses of transcriptomes with a strongly extended taxon sampling (ca. 130 terminals including strepsipterans and neuropterids). Using our morphological data set, this requires considerably more evolutionary steps, 411 (instead of 402) with either Adephaga (Misof *et al.*, 2014) or Myxophaga (unpubl. transcriptomic analyses) (McKenna *et al.*, 2015) as sister taxon of Archostemata, and 413 with Archostemata as sister group of Adephaga + Myxophaga as suggested by Kukalová-Peck & Lawrence (1993, 2004).

The evolutionary scenario differs strongly with Polyphaga as sister group of the remaining suborders. It is in fact the most derived subgroup in terms of thoracic structural features including a series of muscle reductions (Beutel & Haas, 2000; Friedrich *et al.*, 2009). An enforced topology with a clade Adephaga + Myxophaga + Archostemata yields three potential synapomorphies for this unit, the absence of cervical sclerites (85.1; also missing in Strepsiptera, vestigial cervical sclerites present in Ommatidae) (24), the absence of M. mesonoto-trochanteralis (M. 69) (128.0; also missing in Strepsiptera, present in outgroup taxa), hind wings with a marginal joint (143.2), the presence of a bending zone in the medial bar of the hind wing (144.1; also present in Scirtoidea), and RA3+4 cut twice by a triangular fold (148.1). Among these arguments, most of them already suggested by Kukalová-Peck & Lawrence (1993, 2004), the marginal joint (Haas, 1998; Haas & Beutel, 2001) appears as a convincing evolutionary novelty, suggesting a simple flexible anterior hind wing margin as a groundplan feature of Coleoptera. The reconstruction of character evolution under this scenario (Mesquite; Maddison & Maddison, 2011) suggests numerous character reversals in Archostemata, especially concerning the pterothorax. This would include a secondarily acquired transverse ridge of the mesoventrite, the re-appearance of elytral window punctures and cuticular tubercles and scales, a secondarily exposed metatrochantin, and the re-acquisition of an entire series of thoracic muscles (e.g. M. noto-sternalis mesothoracis, M. metasterni primus, M. metanototrochantinalis), which are present in the neuropterid outgroup taxa. This interpretation appears less likely than a numerically less parsimonious alternative scenario: preserved plesiomorphic traits in the wood-associated Archostemata, and multiple parallel character transformations in the other suborders. Presumably ancestral archostematan features were not only lost in the three extant suborders Polyphaga, Adephaga and Myxophaga,

but also in extinct groups assigned to Archostemata such as †Schizophoridae, †Catiniidae or †Ademosynidae (Ponomarenko, 1969; Beutel *et al.*, 2008, 2012). A strong and uniform selective pressure was likely linked with the switch from ancestral wood associated habits (e.g. preference for subcortical spaces) to alternative lifestyles, for instance in riparian or aquatic habitats. This is a possible explanation for the independent evolution of similar morphological syndromes in Myxophaga and the two large extant suborders.

A clade comprising Myxophaga and Polyphaga is not supported in recently published molecular phylogenies (e.g. Misof *et al.*, 2014; Bocak *et al.*, 2014; McKenna *et al.*, 2015). Closer affinities of the latter with Archostemata (McKenna *et al.*, 2015) or Archostemata + Adephaga (Misof *et al.*, 2014) imply that presumptive synapomorphies like the lack of a separate protrochantin and a five-segmented larval leg and single larval claws (Lawrence, 1982; Beutel & Haas, 2000) must have evolved independently. The similar feeding apparatus of basal polyphagan lineages (Anton & Beutel, 2004, 2012; Anton *et al.*, 2016; Antunes-Carvalho *et al.*, 2017) and Myxophaga (Anton & Beutel, 2006) is likely ancestral for Coleoptera as pointed out above, and apparently linked with primarily saprophagous feeding habits.

The relationships within the suborders

The taxon sampling of Archostemata, which includes only two families mostly characterized by plesiomorphic features (Ommatidae, Cupedidae) (Beutel *et al.*, 2008; Friedrich *et al.*, 2009), does not allow any conclusions on the intra-subordinal relationships.

The well supported Adephaga display a mixture of specializations associated with predacious habits (e.g. extraoral digestion in larvae and adults) with a relatively unspecialized condition of thoracic sclerites, with an exposed propleuron, free protrochantin, articulated meso- and metaventrites, and a relatively complete pterothoracic muscle set (Larsén, 1966; Beutel & Haas, 2000). As in earlier contributions based on morphology (Beutel & Roughley, 1988; Beutel *et al.*, 2006, 2012), the specialized surface-swimming Gyrinidae are placed as sister group of the remaining adephagan families. They were included in monophyletic Hydradephaga in some molecular studies (Shull *et al.*, 2001; McKenna *et al.*, 2015). However, a basal position was also retrieved in a recent analysis of ultraconserved elements (UCEs) (Baca *et al.*, 2017) and in recent analyses of transcriptomes (unpubl. results). Like in earlier morphology-based analyses (Beutel & Roughley, 1988; Beutel *et al.*, 2012) Haliplidae were placed as sister group of Dytiscoidea, and Geadephaga were retrieved as a monophyletic unit. This is also in agreement with results of Baca *et al.* (2017) based on UCEs and with recent transcriptome analyses (D. McKenna, unpubl. results).

The pattern in Myxophaga is also consistent with earlier morphology-based hypotheses (Beutel *et al.*, 1999). A clade Myxophaga excl. Lepiceridae appears well supported by the presence of spiracular gills and ligular papillae of larvae, and also a

more or less streamlined body in adults and the loss of the transverse ridge on the metaventrite. Presumptive synapomorphies of Sphaeriusidae and Hydroscaphidae are balloon-shaped spiracular gills, semi-entognathous mouthparts, and rows of lancet-shaped setae on the hind margins of the tergites (Beutel *et al.*, 1999). This seemingly plausible morphology-based scenario is not supported by recent molecular analyses, where Hydroscaphidae are basal, and Lepiceridae and Sphaeriusidae sister taxa (McKenna *et al.*, 2015); D. McKenna pers. comm.).

In Polyphaga, the hydrophiloid and staphylinoid terminals are sister taxa and both form a staphyliniform unit. Scirtoidea are monophyletic but are not placed at the base of the suborder, which is suggested by recent analyses of molecular data (Bocak *et al.*, 2014; McKenna *et al.*, 2015). It is apparent that taxon sampling in the megadiverse Polyphaga is too limited. Moreover, it is questionable whether morphological data alone are sufficient to resolve the relationship in such an extremely complex group (see Lawrence *et al.*, 2011).

Morphological versus molecular data

Morphological characters are still an efficient tool to reconstruct phylogenetic relationships, especially when characters of different life stages and organs and body regions are used (e.g. Friedrich *et al.*, 2010; Beutel *et al.*, 2011). Anatomical investigations require specific skills and experience but are certainly less cost intensive than studies based on transcriptomes and genomes (e.g. Niehuis *et al.*, 2012; Misof et al, 2014). Morphological characters provide an independent data set for critical evaluations of results based on molecular data, often referred to as a ‘plausiblity check’ by molecular workers (e.g. Misof *et al.*, 2014; Peters *et al.*, 2014), an approach addressed as reciprocal enlightenment by the systematist and dipterist Willi Hennig. It is evident that only morphological characters permit reconstruction of character evolution on the phenotypic level (Beutel *et al.*, 2011; Peters *et al.*, 2014), also including transformation of developmental features. A major point is that only morphological feaures allow for placement of fossil taxa, at least in invertebrates (e.g. Beutel *et al.*, 2008, 2012). Furthermore, morphological characters can be gathered from museum specimens unsuitable for obtaining molecular data.

Morphological characters are often subject to homoplasy, resulting from similar patterns of selective pressure, as for instance in the case of independent evolution of mandibular sucking channels in larvae of the adephagan families Gyrinidae, Haliplidae and Dytiscidae (Beutel, 1993; Beutel, 1997; Beutel *et al.*, 2012). Another problem can be concerted convergence (Blanke *et al.*, 2012), a phenomenon caused by correlated characters treated as independent features in phylogenetic analyses. Difficulties linked with morphological data sets and their phylogenetic evaluation can be mitigated by the use of a very broad spectrum of characters (e.g. Beutel *et al.*, 2011). However, even the large data set analyzed in the present study (190 characters of adults and immatures) is arguably affected by parallel evolution, especially in the

case of Myxophaga and Polyphaga. A clade comprising the two suborders is seemingly supported here by apomorphies of larvae and adults (see also Beutel & Haas, 2000; Beutel *et al*., 2008), but not in of the recently published analyses of molecular data (e.g. Bocak *et al.*, 2014; Misof *et al.*, 2014; McKenna *et al.*, 2015; see also McKenna, 2016).

The early evolutionary history of Coleopterida

The earliest appearance of Coleopterida in the Lower Permian (286 mya) is suggested by a transcriptomic tree calibrated with fossils (Misof *et al*., 2014: fig. 2), and a lowermost Mississippian (356 mya, credibility interval 375-336 mya) was proposed in a recent study where data and topologies of McKenna *et al.* (2015) were recalibrated with fossils (Toussaint *et al.*, 2017). However, at present no fossils documenting the earliest evolution of the lineage are available. The oldest known representatives of Strepsiptera are from Burmese amber (Grimaldi *et al.*, 2005; Engel *et al.*, 2016; Pohl & Beutel, 2016), approximately 100 million years old, more than 200 million years after the presumptive origin of Coleopterida. The most ancestral representative of the order – †*Protoxenos* Pohl, Beutel & Kinzelbach (Pohl *et al.*, 2005) – is similar to extant strepsipterans like all other described fossils of this group (e.g. Engel *et al.*, 2016; Pohl & Beutel, 2016). Extinct forms sharing features of both orders and possibly belonging in the stemgroup of Coleopterida are presently unknown.

A crucial event in the evolution of Coleopterida was the development of a posteromotoric flight apparatus. However, this resulted in two very different evolutionary "strategies" in both lineages. In contrast to the protective elytra of beetles, the forewings of Strepsiptera were transformed into halteres, strikingly similar to those of the anteromotoric Diptera (and the Cretaceous Dipteromantispidae; e.g. Makarkin *et al*., 2013) and contributing to the excellent flying abilities of the males as gyroscopic balance organs (Pix *et al.*, 1993). The body was light, with a weak degree of sclerotization and a simplified but efficient pterothoracic muscle apparatus (Koeth *et al.*, 2012). A dense vestiture of microtrichia on the cuticle likely improves the aerodynamic properties of the body surface (Pohl & Beutel, 2008). A lack of mechanical protection was apparently irrelevant for the short-lived males with their advanced flight capacity and high maneuverability. A crucial question in this context is whether endoparasitim of larvae, the related flightlessness of females, and the short adult life span of males (Kinzelbach, 1979;
Pohl & Beutel, 2008) evolved in an early or later stage of strepsipteran evolution. However, presently no information on this issue is available.

In contrast to Coleopterida, the early evolutionary history of Coleoptera is comparatively well documented in the fossil record (e.g. Ponomarenko, 1969, 1977, 1983, 1995; Beutel & Kukalová-Peck, 2012; Yan *et al.*, 2017a-c; see also Crowson, 1975; Kukalová-Peck, 1991; Rasinitsyn & Quicke, 2002) (Figs 2B, 5), with reliable earliest representatives from the Lower Permian (e.g Ponomarenko, 1969, 1995). All

findings of more ancient Carboniferous "beetles" apparently belong to other insect orders. †*Adiphlebia*, a fossil from the Late Carboniferous (Middle Mississippian) of the USA, was interpreted as the earliest known (Béthoux, 2009). However, this assignment was later refuted (Beutel & Kukalová-Peck, 2012), based on lacking synapomorphies with Coleoptera and a venation showing affinities with that of extant Neuroptera. Another extinct taxon from the Upper Carboniferous, †*Stephanastus* Kirejtshuk & Nel, was placed in Coleopterida as a new monotypic order †Skleroptera (Kirejtshuk & Nel, 2013). The authors point out close phylogenetic affinities with †Umenocoleoidea. The arguments for placing †*Stephanastus* within Coleopterida are unspecific (e.g. small trochanters, hidden coxae), preservation-dependent, and insufficiently documented or not visible at all. The wing venation does not support a placement in Coleopterida, but rather suggests affinities with extinct polyneopteran lineages, such as †Protelytroptera (Haas & Kukalová-Peck 2001; Rasnitsyn & Quicke, 2002). A close affinity of †Umenocoleoidea with Coleoptera was also suggested by Kirejtshuk & Nel (2013), without presenting specific evidence. However, this group of "roachoids" belongs to Dictyoptera (Vršanský, 2003), and is apparently closely related with the recently described †Alienopteridae (Bai *et al.*, 2016), both characterized by leathery tegmina and a pronotum transversely subdivided by a supracoxal furrow. Close affinities of the latter group with Mantodea is supported by detailed evidence, including specific features of the head, thorax and genitalia (Bai *et al.*, 2016).

In clear contrast to their sister group, Coleoptera evolved a heavily sclerotized exoskeleton without exposed membranes, with mechanical protection as an obvious benefit, but more or less strongly reduced flying abilities as evolutionary costs. They likely specialized very early on in the penetration of narrow spaces, especially under bark of conifer trees, which probably also provided shelter and food for the larvae (Beutel, 1997). Associated with this wood-associated lifestyle, a prognathous and wedge-shaped head evolved, and also sclerotized elytra covering the dorsal side of the abdomen and the upper pleural regions and terga of the pterothorax. The most ancestral beetles, †Tshekardocoleidae and †Moravocoleidae (†Protocoleoptera) (Beutel & Haas, 2000; Ponomarenko, 1969, 1995; Beutel & Kukalová-Peck, 2012), were characterized by elytra longer and broader than the abdomen, with window punctures and vestiges of original longitudinal veins, a tuberculate cuticle, a broad prosternal process, a broad prothoracic postcoxal bridge, and possibly 13-segmented antennae (Ponomarenko, 1969; Beutel, 1997). Character transformation in the coleopteran stemgroup included the formation of elytra adapted in shape to the abdomen, thus forming a largely closed subelytral space, the narrowing of the prosternal process, the loss of the postcoxal bridge, and possibly the loss of two terminal antennomeres (Beutel, 1997). Plesiomorphies maintained by the extant archostematan families Ommatidae and Cupedidae, but not in any members of the other three suborders are the tuberculate cuticular surface, the elytral window punctures, the transverse ridge on the mesoventrite, the exposed metatrochantin, and a

relatively complex set of thoracic muscles (Beutel & Haas, 2000; Friedrich *et al.*, 2009).

The original reticulate pattern of the elytra was formed by multiplied cross-veins, resulting in a veinal pattern similar to an 'archedictyon', dividing the wing membrane into numerous cells or window punctures. With a reduction in size, the cells were increasingly transformed into vertical supporting structures - columellae. The bottom of these cylindrical (or in some cases conical) structures was composed of the wing membrane, whereas its wall involved the walls of surrounding veins (Krüger, 1898; Ponomarenko, 1969, 1983).

Whereas a presumably wood-associated ancestral life style was maintained in Archostemata, distinct transitions apparently took place in the Upper Permian and Triassic (Fig. 5A, B). A recently discovered adephagan fossil, probably belonging to the stemgroup of Gyrinidae, documents an early invasion of aquatic habitats, with specializations such as subdivided compound eyes and a lobe-shaped pedicellus (E. Yan, pers. obs.). An independent invasion of the aquatic environment was probably accomplished in the same period by †Triaplidae (Ponomarenko, 1977, 2016), in very distinct contrast to Gyrinidae characterized by very large metacoxal plates. Later aquatic or semiaquatic groups are the extinct †Catiniidae and †Schizophoridae (Ponomarenko, 1969, 1983), and possibly also †Ademosynidae, which are possibly close to Polyphaga (Yan *et al.*, 2017a). The recently described family †Peltosynidae (Fig. 5A), assigned to the stemgroup of polyphagans, was certainly terrestrial and likely specialized on wood as suggested by the large and robust mandibles with molae (Yan *et al.*, 2017b).

Recent investigations of beetle fossils underlined that crucial events in the order took place in the late Permian, prior to the Permian-Triassic mass extinction, which affected beetles (and insects in general) less than other groups of organisms. †*Ponomarenkia* Yan, Lawrence, Beattie, Beutel 2017 (Yan *et al.*, 2017c) (Figs. 2B, 5B), one of the very rare Australian beetle fossils of this period (usually only represented by isolated elytra), apparently representing a transitional stage in the group (Fig. 2B). It probably belongs to the crown group of Coleoptera, but in none of the four suborders (Yan *et al.*, 2017c).

The discovery of well-preserved and reliably placed fossils of the stemgroup of Coleopterida would be as desirable as it appears unlikely. Nevertheless, intensive surveys of Carboniferous, Permian, and also Mesozoic fossils should have high priority. The investigation of tremendously rich but incompletely explored fossil materials (e.g. in Russian, South African and Chinese collections) with modern approaches should be intensified. This does not only concern impression fossils of earlier periods, but also Burmese or even Baltic amber, where evolutionary 'leftovers' like †*Protoxenos* may be preserved. New findings may facilitate the understanding of the apparent conflict between results based either on morphological or molecular evidence. Even though the placement of Strepsiptera in Coleopterida appears to be

clarified with different sources of evidence, the early evolution of Coleopterida and Coleoptera is obviously still shrouded in mystery.

Conclusions

The position of Strepsiptera was a matter of long controversy in systematic entomology (Kinzelbach, 1971; Whiting *et al.*, 1997; Wheeler *et al.*, 2001; Wiegmann *et al.*, 2009; Beutel *et al.*, 2011; see also Pohl & Beutel (2013) and Kjer *et al.* (2016). It appears now convincingly solved with analyses of large molecular data sets (Niehuis *et al.*, 2012; Peters *et al.*, 2014; Misof *et al.*, 2014; Boussau *et al.*, 2014) and morphological characters (Friedrich *et al.*, 2010; Beutel *et al.*, 2011). A sister group relationship with a monophyletic Coleoptera is strongly supported by the data presented here. In contrast, the subordinal relationships in Coleoptera remain a challenge, with morphological and molecular data suggesting distinctly different branching patterns. It is conceivable that a denser taxonomic sampling in analyses of transcriptomic data may help to solve this problem. What is suggested by recent analyses of single genes (McKenna *et al.*, 2015) or transcriptomes (e.g. Misof *et al.*, 2014) is clearly in conflict with morphological evidence. The potential of morphological characters appears largely exploited, with well-documented data for different life stages and body regions. In contrast, the exploration of rich fossil sources, especially in collections and fossil sites in Russia and China, has probably still a great potential, as shown by recent discoveries of two new families from the late Permian (Yan *et al.*, 2017c) and late Triassic (Yan *et al.*, 2017b). The knowledge of hitherto unknown fossils belonging to the stem group of Coleopterida would probably lead to a better understanding of the evolution of Holometabola. Extinct groups like † Catiniidae or † Schizophoridae, but also recently described new coleopteran taxa (e.g. Yan *et al.*, 2017a-c), would likely help to reconstruct early splitting events in Coleoptera, which took place before the Permian-Triassic mass extinction.

Supplementary Material

Supplementary material 1. Complete data matrix: Coleopterida_190charFin.nex

Supplementary material 2. Reduced data matrix 1: Coleopterida_156charFin.nex

Supplementary material 3: Fig. A1. Bayesian tree. 190 characters. Posterior probabilities above branches.

Supplementary material 4: Fig. A2. Bayesian tree. 156 characters. With 34 characters excluded, all of them presumptive autapomorphies of Strepsiptera (chars. 1, 13, 16, 29, 33, 35, 37, 40, 42, 50, 53, 56, 59, 63, 67–69, 75, 77, 83, 84, 107, 145, 146, 152, 165, 175, 176, 184–188, 190). Posterior probabilities above branches.

Supplementary material 5: Fig. A3. Bayesian tree. 190 characters. Monophyletic Coleoptera enforced.

Supplementary material 6: homology of thoracic muscles

Acknowledgements

Helpful comments on this manuscript and technical support by Dr. B. Wipfler (Institut für Spezielle Zoologie und Evolutionsbiologie, FSU Jena). The project could be carried out in the framework of the Big4 project (EU). S.-P. Liu was supported from funds of the Big4 project (Marie-Sklodowska-Curie Innovative Training Networks, EU) and E. Yan by the Alexander-von-Humboldt Foundation. All this is gratefully acknowledged.

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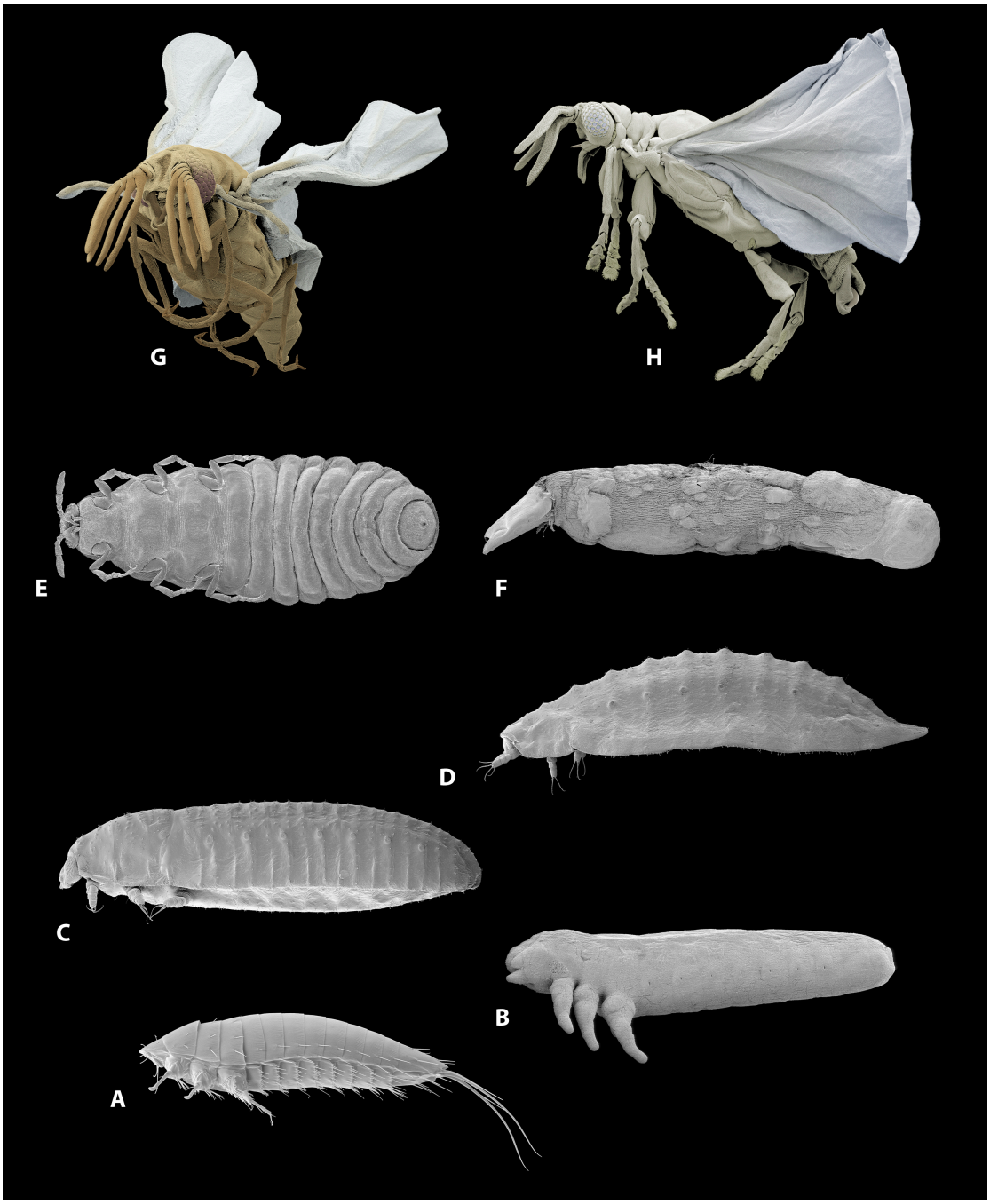

Fig. 1. Strepsiptera, different life stages. **A** Free living first instar larva of *Eoxenos laboulbenei*, lateral view. **B** Endoparasitic secondary instar larva of *E. laboulbenei*, lateral view. **C** Free living male tertiary larva of *Mengenilla chobauti*. **D** Free living female tertiary larva of *M. chobauti*. **E** Free living female of *E. laboulbenei*. **F** Endoparasitic female of *Xenos vesparum*. **G** Male of *M. moldrzyki*. **H** Male of *X. vesparum*. Not to the same scale.

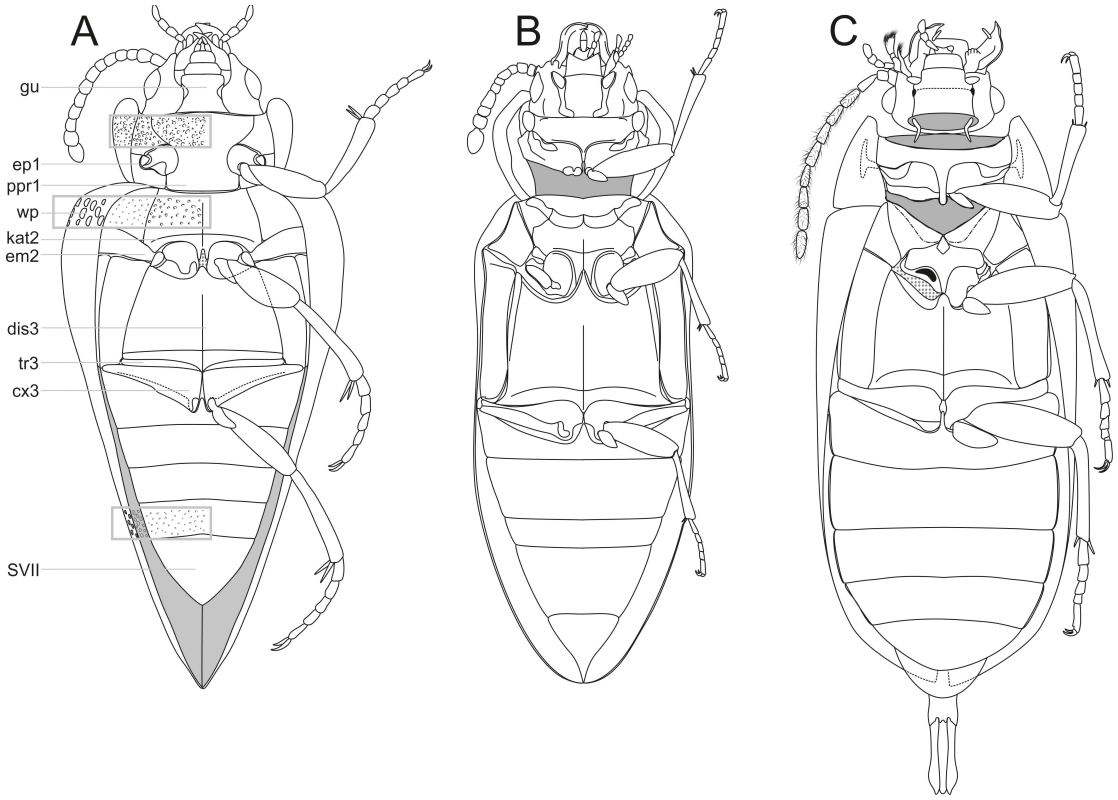

Fig. 2. Hypothesized groundplan conditions and †*Ponomarenkia* (modified from Yan *et al*., 2017a-c). **A**, hypothetical groundplan of Coleoptera s.l. (including stem group): tuberculate surface sculpture; elytrae with window punctures, not closely fitting with abdomen, distinctly projecting beyond abdominal apex; broad prosternal process; procoxal cavities closed, with broad postcoxal bridge; transverse ridge of mesoventrite present; metatrochantin exposed; metacoxae transverse, lacking metacoxal plates; five exposed abdominal ventrites; **B**, †*Ponomarenkius* (Coleoptera incertae sedis, †Ponomarenkiidae), close to hypothetical groundplan of crown group of Coleoptera (Yan *et al*., 2017b): cuticular surface without tubercles or scales; elytra evenly sclerotized, not projecting beyond abdominal apex; propleura exposed; prosternal intercoxal process narrow; procoxal cavities open posteriorly; transverse ridge of mesoventrite present, metatrochantin internalized; **C**, hypothesized groundplan of Polyphaga (Yan *et al*., 2017a, b): cervical sclerites present; propleura internalized; transverse ridge of mesoventrite absent; metatrochantin internalized; aedeagus trilobed.

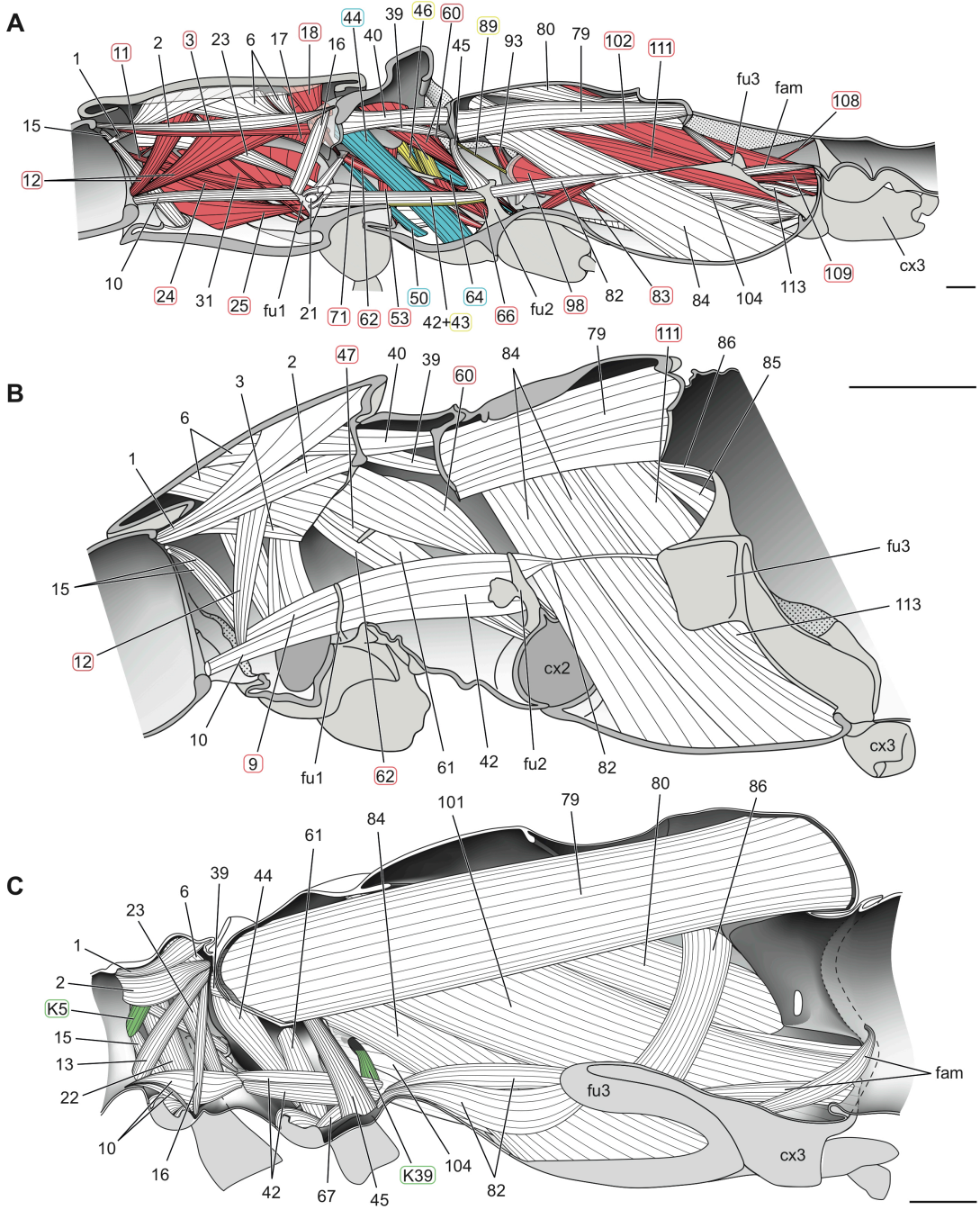

Fig. 3. Comparison of the thoracic muscular system of representatives of **A**, Archostemata (*Tetraphalarus*, Ommatidae; modified from Beutel & Haas, 2000), **B**, Polyphaga (*Helophorus*, Helophoridae; redrawn from Randolf et al., 2013) and **C**, Strepsiptera (*Mengenilla*, Mengenillidae; modified from Beutel & Pohl, 2005). Muscle numbers are based on Beutel & Haas (2000) and Friedrich & Beutel (2008). Muscles present in Archostemata (plesiomorphies), but absent Polyphaga and Strepsiptera (convergent loss) are colored in yellow. Muscles in red and blue are lacking in Strepsiptera or Polyphaga, respectively. Muscles in green in *Mengenilla* are absent in Coleoptera, but likely groundplan features of Coleopterida. Note that muscle

losses are largely restricted to the mesothorax in Coleoptera, whereas a similar degree of reduction affects all thoracic segments in strepsipterans.

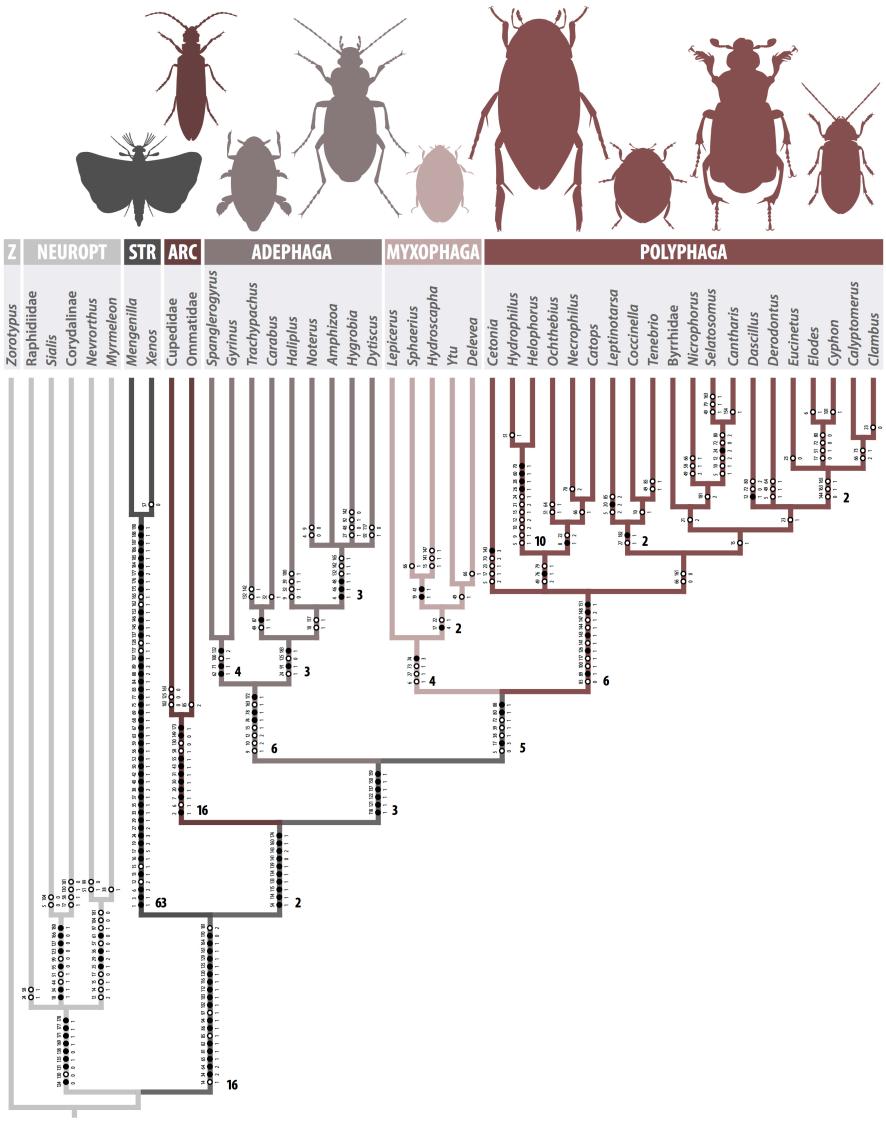

Fig. 4. Phylogeny based on parsimony analysis of 190 morphological characters. Strict consensus of 12 trees (402 steps, CI: 058) obtained with TNT. Unambiguous apomorphies mapped on branches (filled circles non-homoplasious). Branch support values (see list of apomorphies).

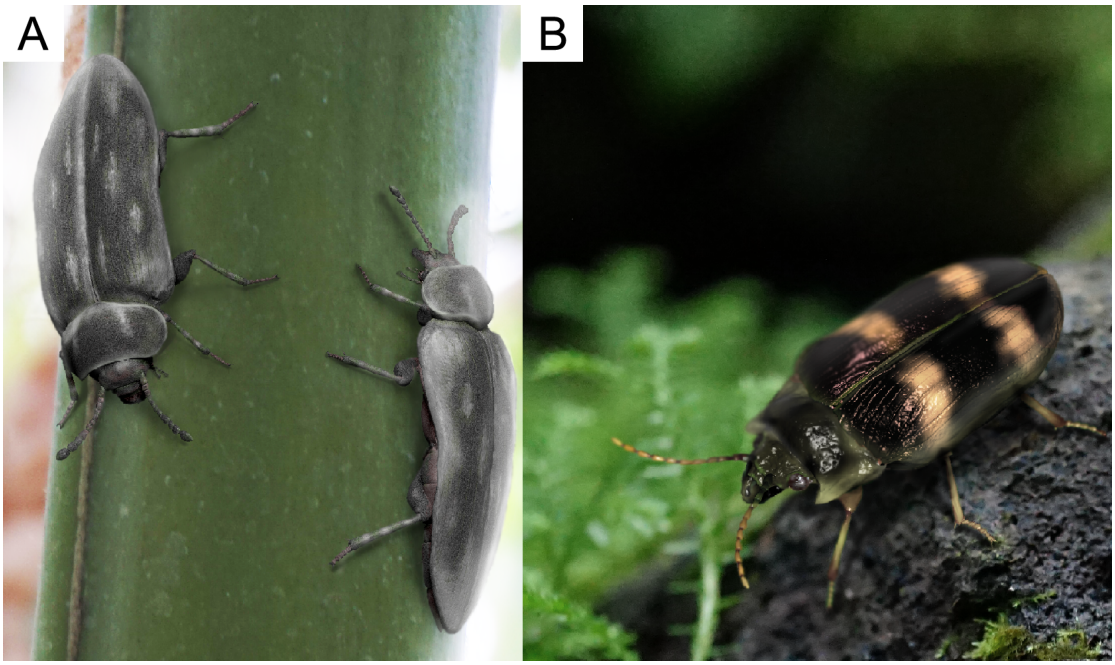

Fig. 5. Late Permian and Late Triassic Coleoptera, habitat reconstruction (from Yan *et al*., 2017b, c, modifed). **A**, †*Ponomarenkia* (Ponomarenkiidae); **B**, †*Peltosyne* (Peltosynidae).

3.5.2. Published Version

Systematic Entomology

Systematic Entomology (2018), DOI: 10.1111/syen.12316

The phylogeny of Coleopterida (Hexapoda) – morphological characters and molecular phylogenies

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Abstract. Coleopterida (Coleoptera + Strepsiptera) has been established as the sister group of Neuropterida (Megaloptera + Neuroptera + Raphidioptera) based on recent phylogenetic analyses of DNA sequence data obtained from genomes and transcriptomes. However, within the resulting clade (Neuropteroidea) the proposed sister-group relationship between the highly specialized endoparasitic Strepsiptera and the megadiverse Coleoptera still lacks convincing morphological support. Furthermore, relationships among the four suborders of Coleoptera remain controversial, with morphological characters strongly conflicting with results suggested by molecular evidence. A large morphological dataset comprising external and internal features of adults and immature stages is presented here and analysed phylogenetically. Our study is focused on deep splits in Coleopterida and on reconstructing character evolution on the phenotypic level. Parsimony analyses clearly support a sister-group relationship between Strepsiptera and monophyletic Coleoptera. Presumptive synapomorphies are characters linked with posteromotorism, but also features of the head and prothorax. We recover Archostemata as sister group of the remaining extant Coleoptera, and Polyphaga as sister group of the species-poor suborder Myxophaga. The most important character complex of Coleoptera is heavy sclerotization without exposed membranes and a simplification of the thoracic muscle apparatus. Non-archostematan beetles are characterized by further simplifications of the thoracic locomotor apparatus. This trend reaches its peak in Myxophaga and Polyphaga, and these suborders also share apomorphies of the larval legs. A pattern with Polyphaga as sister to all other suborders and a clade Myxophaga + Archostemata (as in recent molecular phylogenetic studies) requires ten additional steps with our dataset. This scenario implies that various simplifications of the thoracic exoskeleton and musculature have taken place several times independently, and also that a complex feeding apparatus suitable for saprophagy and sporophagy was ancestral in Coleoptera, with secondary reduction (or modification) in Archostemata and Adephaga. The coleopteran subordinal relationships remain a challenge, with morphological and molecular data suggesting distinctly different patterns. The earliest evolution of Coleopterida is not documented in the fossil record. The exploration of potential stem-group fossils is a high priority, as is the study of species from the Permian–Triassic transition zone, which are apparently important in the context of evaluating the relationships among beetle suborders.

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Introduction

With about 350 000–400 000 described extant species, beetles comprise roughly 25% of the diversity of all organisms (Stork *et al.,* 2015). In contrast, the highly specialized endoparasitic Strepsiptera (Fig. 1) are a very small group, with only slightly more than 600 known extant and extinct species (e.g. Kinzelbach, 1971; Pohl & Beutel, 2008). A close relationship between these two holometabolous orders was suggested before molecular approaches were used, with Strepsiptera proposed either as the sister taxon of monophyletic Coleoptera (Kinzelbach, 1971; Beutel & Gorb, 2001) or as a subordinate and secondarily highly modified polyphagan subgroup (Crowson, 1955, 1981). Some molecular phylogenetic analyses based exclusively on parsimony analyses of ribosomal DNA sequences suggested a group including Strepsiptera and Diptera (Halteria) (Whiting *et al.,* 1997; Wheeler *et al.,* 2001). This concept has not been supported by recent investigations based on morphology (Friedrich & Beutel, 2010; Beutel *et al.,* 2011), single-copy nuclear genes (Wiegmann *et al.,* 2009; McKenna & Farrell, 2010; Ishiwata *et al.,* 2011), genomes (Niehuis *et al.,* 2012; McKenna, 2014), and transcriptomes (Boussau *et al.,* 2014; Misof *et al.,* 2014; Peters *et al.,* 2014). Monophyletic Coleoptera as the sister taxon of Strepsiptera is supported by most recent molecular phylogenetic studies (e.g. Boussau *et al.,* 2014; Misof *et al.,* 2014; Peters *et al.,* 2014; McKenna *et al.,* 2015). However, Coleopterida was only vaguely supported by morphological arguments to date, with most or all potential synapomorphies related to a single character complex, posteromotorism (Friedrich & Beutel, 2010; Beutel *et al.,* 2011). Moreover, character transformations in the exceedingly heterogenous Coleopterida remained obscure. The crucial issue of the basal splitting events in Coleoptera is not convincingly solved yet (Beutel & McKenna, 2016; McKenna, 2016), with either Archostemata (Beutel & Haas, 2000; Beutel *et al.*, 2008a; Friedrich *et al.,* 2009) or Polyphaga (Kukalová-Peck & Lawrence, 1993, 2004; Misof *et al.,* 2014; McKenna *et al.,* 2015) placed as the sister group of the remaining suborders. A clade comprising the small suborders Archostemata and Myxophaga was recovered in recent analyses of molecular data (McKenna *et al.,* 2015), despite lacking morphological synapomorphies and striking differences in their habitus and lifestyle (e.g. Reichardt, 1973; Hörnschemeyer, 2005).

Investigations of the morphology and phylogeny of the neuropteroid branch of Holometabola (Neuropterida, Strepsiptera, Coleoptera) have made impressive progress in the last two decades. This has been facilitated by new technologies accelerating the acquisition of high-quality anatomical data (Friedrich *et al.,* 2013; Wipfler *et al.,* 2016). Numerous studies were published on different life stages and body regions of Neuropterida (Aspöck & Aspöck, 2008; Beutel *et al.*, 2008b, 2010a,b), Coleoptera (Anton & Beutel, 2004, 2006, 2012; Beutel & Komarek, 2004; Friedrich & Beutel, 2006; Beutel *et al.*, 2008a; Friedrich *et al.,* 2009; Lawrence *et al.,* 2011; Anton *et al.,* 2016; Antunes-Carvalho *et al.,* 2017) and Strepsiptera (Beutel & Pohl, 2005; Pohl & Beutel, 2005, 2008; Osswald *et al.,* 2010; Koeth *et al.,* 2012; Fraulob *et al.,* 2015; Knauthe *et al.*, 2016). Moreover, extensive morphological datasets were compiled for the entire Holometabola (Friedrich & Beutel, 2010; Beutel *et al.,* 2011). Considering the wealth of morphological information available now and new phylogenetic results based on molecular data, it appeared appropriate to extend and improve previously analysed data matrices (Beutel & Haas, 2000; Beutel *et al.*, 2008a; Friedrich *et al.*, 2009), and present them here as a future resource for phylogenetic studies of this important group of arthropods. The compiled dataset of 190 characters of immature stages and adults was analysed with maximum parsimony and Bayesian inference. The results are discussed with respect to recent phylogenies based on eight individual genes (Wiegmann *et al.,* 2009) and transcriptomes (Misof *et al.,* 2014). Possible scenarios of character evolution are evaluated under different phylogenetic patterns. The fossil record is discussed with respect to Coleopterida and early splitting events among beetles.

Methods

Taxon sampling

The taxon sampling is similar to that of Friedrich *et al.* (2009), but with the addition of Nevrorthidae (Neuroptera), *Lepicerus* Motschulsky (Lepiceridae, Myxophaga), and two terminals of Strepsiptera, *Xenos* Rossius and *Mengenilla* Hofeneder, the former representing a species-rich family and the latter Mengenillidae, a family mostly characterized by plesiomorphic features (Pohl & Beutel, 2005, 2008). *Micromalthus* LeConte was excluded as a specialized member of Archostemata with many autapomorphies, but features of this unusual taxon (e.g. Hörnschemeyer, 2005) are provided in the list of characters. Specimens, scanning electron microscopy micrographs, microtome sections and microcomputed tomography datasets of species listed in Friedrich *et al.* (2009) were used for this contribution.

The core of the morphological character set is based on studies of Beutel & Haas (2000), Beutel *et al.* (2008a) and Friedrich *et al.* (2009). Additional data were extracted from recent contributions on outgroup and ingroup taxa, including an extensive and well-documented dataset for the entire Coleoptera (Lawrence *et al.,* 2011), investigations on the wing venation (Kukalová-Peck & Lawrence, 1993, 2004), and studies on Neuropterida (Beutel *et al.,* 2010a,b; Zimmermann *et al.,* 2011; Randolf *et al.,* 2014), the cephalic morphology of Coleoptera (Dressler & Beutel, 2010; Anton *et al.,* 2016; Antunes-Carvalho *et al.,* 2017), and the anatomy of the thorax (Friedrich & Beutel, 2006; Friedrich *et al.,* 2009; Koeth *et al.,* 2012). More detailed information on selected taxa, fixation and applied techniques is provided in previous studies (Friedrich *et al.,* 2009; Friedrich & Beutel, 2010; Beutel *et al.,* 2011; see also Wipfler *et al.,* 2016 for a review of techniques).

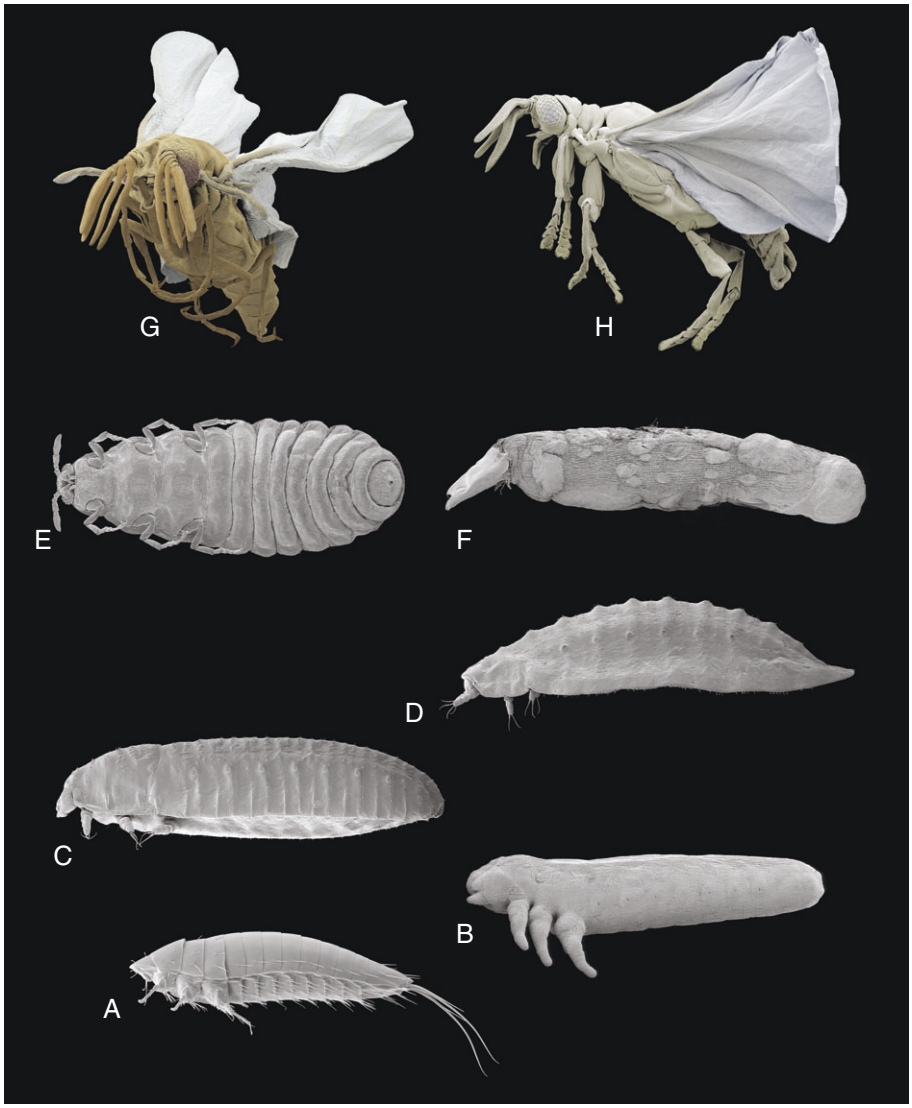

Fig. 1. Strepsiptera, different life stages. (A) Free-living first-instar larva of *Eoxenos laboulbenei*, lateral view. (B) Endoparasitic secondary instar larva of *E. laboulbenei*, lateral view. (C) Free-living male tertiary larva of *Mengenilla chobauti*. (D) Free-living female tertiary larva of *M. chobauti*. (E) Free-living female of *E. laboulbenei*. (F) Endoparasitic female of *Xenos vesparum*. (G) Male of *Mengenilla moldrzyki*. (H) Male of *X. vesparum*. Not to the same scale. [Colour figure can be viewed at wileyonlinelibrary.com].

Cladistic analysis

Data were entered in a matrix using WINCLADA (Nixon, 1999). Parsimony analyses (all characters with equal weight and unordered) were carried out with NONA (ratchet, 1000 replications) (Goloboff, 1995) and TNT (traditional search, random seed 1, 10 repl., TBR, ten trees saved per replication) (Goloboff *et al.,* 2008). Bremer-support values (Bremer, 1994) were calculated with NONA. *Zorotypus* Silvestri sp. (Zoraptera), Raphidiidae, *Myrmeleon* Linnaeus sp., *Nevrorthus* Costa sp., Corydalinae, and *Sialis* Latreille sp. were used as outgroup taxa and treated as all other groups in the analysis (simultaneous analysis; Nixon & Carpenter, 1993). Bayesian analyses were performed with MRBAYES 3.2.6 (Huelsenbeck & Ronquist, 2001, 2003; see also Lewis, 2001). The standard model for variable morphological characters (Mkv model; Huelsenbeck & Ronquist, 2003) was used with gamma for state frequencies and 0.1 for temperature. Four simultaneous runs of five million generations were conducted, each with one cold and three heated chains. Samples were drawn every 500 Markov chain Monte Carlo steps, with the first 25% discarded as burn-in. The run was automatically stopped when the average standard deviation of split frequencies was below 0.01. The analyses were carried out with a full dataset of 190 characters and with a reduced set with 34 characters excluded, all of them presumptive autapomorphies of Strepsiptera (see Figures S1 and S2).

List of morphological characters

The names and numbers of the cephalic muscles were taken from Kéler (1963) and Dressler & Beutel (2010). Numbers for thoracic muscles are based on Beutel & Haas (2000) (and homologized with a generalized muscle list for Neoptera; Friedrich & Beutel, 2008) (see also Table S1 and Beutel *et al.,* 2014).

The data matrices in Nexus format are provided as electronic supplements (see Files S1 and S2): Coleopterida_190char.nex (full dataset with 190 characters), Coleopterida_156char.nex [reduced dataset with 34 characters (presumptive autapomorphies of Strepsiptera) removed].

Larvae, general

1. Size of first instar: (0) > 0.3 mm; (1) < 0.3 mm. Primary larvae of Strepsiptera (first instar) are on average *c*. 0.2 mm long and the minimum length is 0.07 mm (Pohl, 2000; Knauthe *et al.,* 2016) (Fig. 1A). A very small size can also be assumed for primary larvae of *Sphaerius*, but it is unlikely that they reach a similar degree of miniaturization. The penultimate instars are 0.84–1.20 mm long according to Britton (1966) [see also Beutel & Arce-Pérez, 2016: fig. 6.6D)] (coded as 0). Primary larvae of the endoparasitic Rhipiphoridae are also larger than those of Strepsiptera (Lawrence *et al.* 2010) and far-reaching effects of miniaturization described for Strepsiptera (Knauthe *et al.*, 2016) are unknown in Coleoptera (Reichardt, 1973; Hörnschemeyer, 2005).

Larvae, head

2. Dorsal endocarina: (0) absent; (1) present. Well developed in Archostemata (Beutel & Hörnschemeyer, 2002a,b) and some groups of Polyphaga not included here (Lawrence *et al.*, 2011). The occurrence in larvae of some scattered polyphagan families is apparently the result of parallel evolution. The endocarina is generally lacking in Scirtoidea and Staphyliniformia (Beutel & Leschen, 2005; Lawrence *et al.*, 2011).
3. Larval tentorium: (0) present; (1) absent. Completely reduced in larvae of Strepsiptera (Pohl, 2000; Müller, 2013; Pohl & Beutel, 2013; Knauthe *et al.*, 2016).
4. Caudal tentorial arms: (0) absent or short; (1) elongated, attached to posteroventral part of head capsule. Elongated and posteriorly connected with head capsule in Dytiscoidea excluding Noteridae (Beutel, 1993; Beutel *et al.*, 2013). Coded as inapplicable for Strepsiptera.
5. Orientation of head: (0) subprognathous, slightly to moderately inclined; (1) prognathous or hyperprognathous; (2) orthognathous. Prognathous in Strepsiptera (Pohl, 2000), Adephaga (Beutel, 1993), Archostemata (Beutel & Hörnschemeyer, 2002a,b), Hydrophiloidea (excluding Spercheidae) (Beutel, 1999) and some other groups of Polyphaga (Elateroidea, Cantharoidea, Cleroidea) (Beutel, 1995; Beutel & Pollock, 2000). Orthognathous in Scarabaeoidea and most groups of Chrysomeloidea (Lawrence *et al.*, 2011; Scholtz & Grebennikov, 2016).
6. Head shape of later instars: (0) not transverse, not strongly rounded laterally; (1) transverse, broader than long, strongly rounded laterally; (2) globular and simplified. Transverse in later instars of Archostemata (Beutel & Hörnschemeyer, 2002a,b) and Myxophaga (Beutel *et al.*, 1999), even though less distinctly in the presumptive larva of *Lepicerus* (Lawrence *et al.*, 2013). Also transverse in some Scirtidae (LeSage, 1991; Lawrence, 2016; coded as 0&1). Globular and strongly simplified in endoparasitic secondary larvae of Strepsiptera (Müller, 2013).
7. Deep dorsal and ventral posteromedian emargination: (0) absent; (1) present. Present in Archostemata (Beutel & Hörnschemeyer, 2002a,b).
8. Hemispherical projection of head capsule between mandibular and maxillary articulation: (0) absent; (1) present. Present in Hydraenidae, Leiodidae, Agyrtidae and Ptiliidae (Beutel & Leschen, 2005).
9. Posterior tentorial grooves: (0) close to hind margin of head; (1) shifted anteriorly. Distinctly shifted anteriorly in Nevrorthidae, Raphidioptera, Corydalidae (Beutel & Friedrich, 2008), and several groups of Coleoptera, especially in Adephaga, Hydrophiloidea and Staphylininae (Beutel, 1993, 1999; Beutel & Molenda, 1997).
10. Gula: (0) absent or not recognizable as a defined sclerotized element; (1) undivided sclerotized quadrangular gula; (2) strongly narrowed gula. Well-developed and undivided larval gula present in Raphidioptera (Beutel *et al.*, 2008b), Corydalidae (Beutel & Friedrich, 2008), Nevrorthidae (Beutel *et al.*, 2010a), and Coleoptera (partim; Lawrence *et al.*, 2011). Strongly narrowed in some groups of Adephaga (Gyrinidae, Trachypachidae, Carabidae major part) and most groups of Hydrophiloidea (Beutel, 1993, 1999). The short and indistinctly defined gula of *Zorotypus* is scored as 0. The posteroventral sclerite of the head capsule of larval Nevrorthidae is a gula in terms of position and specific quality (Beutel *et al.*, 2010a), even though recent results of molecular studies (Wang *et al.*, 2017; Winterton *et al.*, 2018) suggest that it may have evolved independently and is a potential autapomorphy the family.
11. Number of retinula cells in ommatidia or stemmata: (0) < 15; (1) ≥ 15. Number strongly increased in Neuropterida, especially in Megaloptera where up to 40 retinula cells can be present (Paulus, 1986).
12. Articulation of labrum: (0) free; (1) partly fused; (2) completely fused. Fused in Strepsiptera (Pohl, 2000), Adephaga and Hydrophiloidea (Beutel, 1993, 1999), and also in some other groups of Polyphaga (e.g. Cantharidae; Beutel, 1995). Partly fused in Dascillidae (Lawrence *et al.*, 2011).
13. Anterior margin of head capsule; (0) not forming sharp cutting edge; (1) forming sharp cutting edge. The wedge-shaped head of primary larvae of Strepsiptera is characterized by a sharp anterior cutting edge (Knauthe *et al.*, 2016) (Fig. 1A). A rounded or toothed anterior margin of the clypeolabrum occurs in some groups of beetles with prognathous and predacious larvae (e.g. Adephaga,

Hydrophiloidea; (Beutel, 1993, 1999). However, it does not form a cutting edge suitable for penetrating the body wall of an insect.

14. M. frontolabralis (M. 8) of later instars: (0) present; (1) absent. Present in *Raphidia* Linnaeus (Beutel *et al.*, 2008b) and Megaloptera (Röber, 1942; Beutel & Friedrich, 2008), but absent in Neuroptera (Beutel *et al.*, 2010a) and Strepsiptera (Pohl, 2000; Knauthe *et al.*, 2016). Also absent in Coleoptera (Beutel, 1993, 1999; Beutel & Molenda, 1997; Beutel & Hörnschemeyer, 2002a,b) with the exception of first-instar larvae of *Tenomerga* (Yavorskaya *et al.*, 2015) (scored as 1 for Cupedidae).
15. M. frontoepipharyngalis (M. 9): (0) present; (1) absent. Present in some groups of Coleoptera (e.g. Cupedidae, Myxophaga excluding Hydroscaphidae; Das, 1937; Beutel *et al.*, 1999; Beutel & Hörnschemeyer, 2002a,b; Yavorskaya *et al.*, 2015), in *Raphidia* (Beutel *et al.*, 2008b) and Megaloptera (Röber, 1942; Beutel & Friedrich, 2008), and in some genera of Neuroptera (Wundt, 1961; Rousset, 1966; Beutel *et al.*, 2010a). Absent in Strepsiptera (Knauthe *et al.*, 2016).
16. Antenna of primary larva: (0) distinctly developed; (1) reduced. Reduced in larvae of Strepsiptera, only recognizable as an entirely flat antennal field (Knauthe *et al.*, 2016) (Fig. 1A).
17. Number of antennomeres in last instar: (0) > 13; (1) five; (2) four; (3) three; (4) two; (5) no antennomere recognizable. Multisegmented in larvae of Scirtidae (LeSage, 1991; Lawrence, 2016) and also in some larvae of Neuroptera, possibly related to a secondary subdivision of the penultimate antennomere (Beutel *et al.*, 2010a, coded as 0). Four-segmented in Sialidae (Röber, 1942), Raphidioptera (Beutel *et al.*, 2008b), Cupedidae and Ommatidae (Lawrence, 1982, 1999; Beutel & Hörnschemeyer, 2002a,b), in Adephaga (Beutel, 1993) and in few groups of Polyphaga (Lawrence, 1982). Five-segmented in Corydalidae (Beutel & Friedrich, 2008) and usually also in scarabaeoid larvae (Lawrence *et al.*, 2011). Three-segmented in Chrysopidae (Beutel *et al.*, 2010a), Lepiceridae (Lawrence *et al.*, 2013) and almost generally in Polyphaga (Lawrence *et al.*, 2011). Two-segmented in Myxophaga excluding Lepiceridae and in first-instar larvae of *Tenomerga* Neboiss (Beutel *et al.*, 1999; Yavorskaya *et al.*, 2015). No recognizable antennomeres are present in secondary larvae of Strepsiptera (Pohl, 2000; Knauthe *et al.*, 2016).
18. Sensorium on antepenultimate antennomere: (0) absent; (1) present. Present in larvae of Megaloptera (Beutel & Friedrich, 2008). Not applicable in Strepsiptera.
19. Exposure of mouthparts: (0) exposed; (1) semi-entognathous, labrum laterally fused with triangular genal lobe; (2) mandibles largely internalized, ventral mouthparts partly fused with head capsule. Semi-entognathous in Hydroscaphidae and Sphaeriusidae (Beutel *et al.*, 1999). Mandibles of primary strepsipteran larvae largely internalized, ventral mouthparts strongly modified (Pohl, 2000; Knauthe *et al.*, 2016).
20. Mandibular apex: (0) slender, with one or several pointed teeth; (1) three blunt and strong teeth; (2) blunt, with more than three apical teeth. With three strong apical teeth in Archostemata (Beutel & Hörnschemeyer, 2002a,b; Yavorskaya *et al.*, 2015). More than three teeth in many chrysomelid larvae (e.g. Lawrence *et al.*, 2011).
21. Mandibular mola: (0) present, not quadrangular, not delimited by distinct margin; (1) present, quadrangular, delimited by distinct margin; (2) absent. Present in Archostemata (Beutel & Hörnschemeyer, 2002a,b; Yavorskaya *et al.*, 2015), Myxophaga (Beutel *et al.*, 1999), and many groups of Polyphaga (e.g. Scirtoidea) (Lawrence, 2016). Quadrangular and delimited by a distinct margin in older instars of Cupedidae (coded as 1) and *Micromalthus* (Beutel & Hörnschemeyer, 2002a,b).
22. Prostheca: (0) absent; (1) present, rounded and semimembranous; (2) present, slender. Rounded and semimembranous, with small, posteriorly directed spines in Torridincolidae and Hydroscaphidae (Beutel *et al.*, 1999). Slender, with one or several apices in Hydraenidae, Agyrtidae, Leiodidae (partim), Clambidae, Eucinetidae (coded as absent for Scirtidae), Derodontidae, and others (Lawrence, 1982, 2016; Beutel & Leschen, 2005; Lawrence *et al.*, 2011).
23. Accessory ventral process of mandible: (0) absent; (1) present. Present in Clambidae (partim), Scirtidae, Dascillidae, Scarabaeoidea and Derodontidae (Lawrence *et al.*, 2011; Lawrence, 2016).
24. Intramaxillary movability: (0) fully retained; (1) reduced, not forming functional complex with labium; (2) reduced, maxillolabial complex; (3) Maxillae medially connected. Movability distinctly reduced in Raphidioptera, Adephaga (excluding Gyrinidae), Hydrophiloidea, and Histeroidea (Beutel, 1993, 1999; Beutel & Haas, 2000). Maxillolabial complex present in most groups of Elateriformia (Beutel, 1995) and Cleroidea (Beutel & Pollock, 2000). Medially connected and plate-like in primary larvae of Strepsiptera (Pohl, 2000; Knauthe *et al.*, 2016). Coded as inapplicable for Neuroptera (see char. 25) (Beutel *et al.*, 2010a).
25. Link between mandibles and maxillae: (0): absent; (1) maxillae form sucking jaws with mandibles. The formation of sucking jaws by the longitudinal interlocking of the mandibles and maxillae is a unique feature of Neuroptera (Beutel *et al.*, 2010a).
26. Cardo: (0) not subdivided into several sclerites; (1) subdivided into several sclerites. Subdivided in Hydrophiloidea (Beutel, 1999). Coded as inapplicable for Strepsiptera where a cardo is not present as a defined maxillary element (Knauthe *et al.*, 2016).
27. Separate galea: (0) present; (1) absent. Not present as separate element in Myxophaga, in most subgroups of Staphylinidae, in Clambidae (with the exception of *Calyptomerus* Redtenbacher; coded as 0), and in Cucujiformia (Lawrence, 1982, 2016; Lawrence *et al.*, 2011). Probably forming a mala with the lacinia in these groups. Missing in *Hygrobia* and Strepsiptera (Pohl, 2000; Beutel *et al.*, 2006).

28. Insertion of galea: (0) stipes or unsclerotized proximomesal part of palpifer 1; (1) distal part of palpifer. Inserted on distal part of palpifer in Hydrophiloidea (Beutel, 1999).
29. Maxillary palp: (0) present and composed of several segments: (1) distinctly reduced, bolt-shaped socket and long seta; (2) absent. Maxillary palp and palp muscles absent in Neuroptera (Beutel *et al.*, 2010a). Composed of bolt-shaped socket and long seta in primary larvae of Strepsiptera (Knauthe *et al.*, 2016).
30. Submentum and mentum: (0) not fused and narrowed between maxillary fossae; (2) fused and narrowed between maxillary fossae. Fused and narrowed between maxillary fossae in larvae of Archostemata (Beutel & Hörnschemeyer, 2002a,b; Yavorskaya *et al.*, 2015). Ventral mouthparts highly modified in primary larvae of Strepsiptera (coded as inapplicable).
31. Ligula: (0) not wedge-shaped and enlarged; (1) enlarged and wedge-shaped. Enlarged, sclerotized, and wedge-shaped in Archostemata (Beutel & Hörnschemeyer, 2002a,b; Yavorskaya *et al.*, 2015).
32. M. submentopraementalis (M. 28): (0) present; (1) absent. M. submentopraementalis is absent in primary larvae of Strepsiptera (Knauthe *et al.*, 2016), in larvae of Archostemata (Beutel & Hörnschemeyer, 2002a,b; Yavorskaya *et al.*, 2015), and in some other groups of beetles (Beutel, 1993). It is generally present in Neuropterida (e.g. *Chrysopa* Leach, *Sialis*; Röber, 1942; Wundt, 1961; Beutel & Friedrich, 2008) and also in most groups of Coleoptera (Beutel, 1993, 1995, 1999).
33. External opening of preoral cavity: (0) single opening (functional mouth opening); (1) double opening. Double opening present in primary larvae of Strepsiptera (Pohl, 2000; Knauthe *et al.*, 2016).
34. Larval salivary ducts and glands: (0) well developed; (1) strongly narrowed, without recognizable lumen; (2) absent; (3) tube-like elongated glands without ducts. Narrow, vestigial proximal salivary tube present in Megaloptera (Beutel & Friedrich, 2008). Absent in Nevrorthidae (Beutel *et al.*, 2010a), non-cucujiform Coleoptera (Beutel, 1993, 1995, 1999; Beutel & Molenda, 1997; Beutel *et al.*, 1999; Beutel & Hörnschemeyer, 2002a,b; Yavorskaya *et al.*, 2015) and Strepsiptera (Knauthe *et al.*, 2016). Long tube-like glands are present in Cucujiformia (e.g. Beutel & Pollock, 2000). Their structure is completely different from salivary glands found in other groups of insects.
35. Position of brain: (0) head or anterior prothorax; (1) middle region of posterior postcephalic body. Brain and suboesophageal ganglion shifted to middle region of postcephalic body in primary larvae of Strepsiptera (Beutel *et al.*, 2005).

Larvae, thorax

36. Cervix: (0) absent; (1) present. Distinct separate cervix present in Neuroptera (Beutel *et al.*, 2010a). Absent in all other groups.
37. Sternal plates: (0) absent; (1) present. Sternal plates formed by true sternites inserted between coxae in all primary larvae of Strepsiptera (Pohl, 2000; Osswald *et al.*, 2010).
38. Leg segmentation: (0) six free leg segments; (1) tibia fused with tarsus; (2) Femur fused with trochanter. Six-segmented in Adephaga and Archostemata with well-developed legs (Lawrence, 1982, 1999; Beutel & Hörnschemeyer, 2002a,b). Five-segmented with tibiotarsus in Myrmeleontidae (Sundermeier, 1940), Myxophaga and Polyphaga (Lawrence, 1982; Beutel *et al.*, 1999). Femur fused with tibia in primary larvae of Strepsiptera (Fig. 1A), with a suture still recognizable in Mengenillidae (Pohl, 2000; Osswald *et al.*, 2010).
39. Number of claws: (0) double; (1) single. Single claw in Haliplidae, Myxophaga, Polyphaga, and first-instar larvae of *Priacma* LeConte (coded as 0&1 for Cupedidae) (Lawrence, 1999; Beutel & Hörnschemeyer, 2002a,b; Yavorskaya *et al.*, 2015). Claws absent in Strepsiptera (coded as inapplicable) (Pohl, 2000; Pohl & Beutel, 2004).
40. Lobe-like pretarsal attachment pads: (0) absent; (1) present. Present in primary larvae of Strepsiptera (Pohl, 2000; Pohl & Beutel, 2004), differing distinctly from adhesive devices occurring in larvae of very few groups of Coleoptera (Coccinellidae; coded as 0).
41. Regular rows of lancet-shaped setae on hind margins of thoracic and abdominal terga: (0) absent; (1) present. Present in Hydroscaphidae and Sphaeriusidae (Beutel *et al.*, 1999).

Larvae, abdomen

42. Posterior edges of abdominal sternites: (0) not densely covered with bristles and spinulae; (1) with densely arranged bristles and spinulae. Dense rows of bristles and spinulae present on posterior edges of abdominal sternites of primary larvae of Strepsiptera (Pohl, 2000; Knauthe *et al.*, 2016).
43. Abdominal tergal ampullae: (0) absent; (1) present. Present in Archostemata (Beutel & Hörnschemeyer, 2002a,b; Yavorskaya *et al.*, 2015).
44. Setiferous lateral gill filaments: (0) absent; (1) present. Setiferous lateral tracheal gills are present in larvae of Megaloptera (Aspöck & Aspöck, 2003), Gyrinidae, *Coptotomus* (Dytiscidae), and *Berosus* (Hydrophilidae), and they also occur in a subgenus of *Helophorus* Leach (Helophoridae) (Lawrence *et al.*, 2011).
45. Spiracular gills: (0) absent; (1) present, long and slender; (2) present, short and bulbous. Present in Myxophaga excluding Lepiceridae (Beutel *et al.*, 1999; Lawrence *et al.*, 2013). Short and bulbous in Hydroscaphidae and Sphaeriusidae (Beutel *et al.*, 1999).
46. Abdominal segment IX: (0) well developed, tergum present; (1) largely reduced, tergum absent. Largely reduced in Dytiscoidea with the exception of Aspidytidae (Beutel *et al.*, 2006).
47. Abdominal segment XI: (0) well developed, tergum present; (1) absent. Present in primary larvae of Strepsiptera (Beutel *et al.*, 2011).
48. Size and position of spiracles VIII: (0) not enlarged and terminal; (1) enlarged and terminal. Enlarged in Noteridae, Amphizoidae, and Dytiscidae. Closed and replaced by

ventral gills in Hygrobiidae (coded as 0) (Beutel *et al.*, 2006).

49. Urogomphi: (0) absent; (1) present, fixed; (2) present, articulated. Articulated or fixed urogomphi missing in Archostemata (Lawrence, 1982, 1999; Beutel & Hörnschemeyer, 2002a,b), Hydroscaphidae, and Sphaeriusidae, and also in many groups of Polyphaga (e.g. Scarabaeidae, Scirtoidea, Dascillidae) (Lawrence, 1982, 2016; Lawrence *et al.*, 2011). Movable urogomphi occur in Adephaga and Staphylinoidea (e.g. Beutel & Leschen, 2005; Lawrence *et al.*, 2011).
50. Terminal abdominal jumping apparatus of segment XI: (0) absent; (1) present. Formed by long and strongly developed bristles (cerci) of abdominal segment X in primary larvae of Strepsiptera (Pohl & Beutel, 2004). Jumping capacity secondarily reduced in *Stylops* Kirby (Kinzelbach, 1971).

Larval ecology

51. Larval habitat: (0) terrestrial or semiaquatic; (2) aquatic. Larvae of Nevrorthidae, Sisyridae and Megaloptera are aquatic (e.g. Beutel & Friedrich, 2008; Beutel *et al.*, 2010a; Randolf *et al.*, 2013) and also immatures of different groups of Coleoptera (e.g. Reichardt, 1973; Crowson, 1981; Lawrence, 1982). Larvae of Osmylidae are found in riparian habitats (coded as 0). Aquatic larval development apparently evolved independently in Adephaga (Gyrinidae, Haliplidae, Dytiscoidea), Myxophaga (Torridincolidae, Hydroscaphidae) and several polyphagan subgroups (e.g. Hydraenidae, Hydrophiloidea major part; Elmidae etc.) (Beutel, 1997).
52. Endoparasitism of larvae: (0) absent; (1) present. Immature stages of Strepsiptera are endoparasitic (e.g. Pohl & Beutel, 2005) (Fig. 1B).

Pupal characters

53. Movability of pupal mandible: (0) absent; (1) present. Pupal mandible movable in Neuropterida (Aspöck & Aspöck, 2003) and few other groups of Holometabola. Immobilized in Coleoptera and in contrast to Kinzelbach (1971) also in Strepsiptera.

Adults, general

54. Sclerites: (0) connected by extensive, externally exposed membranes; (1) firmly connected, no membranes exposed externally. Sclerites not covered by elytra closely attached to each other in Coleoptera (with few exceptions), without exposed membranes (Lawrence, 1982; Beutel & Haas, 2000) (Fig. 2).
55. Cuticular surface: (0) without scale-like structures: (1) scale-like structures present. Scale-like surface structures inserted on cuticular tubercles (Fig. 2A) in Cupedidae and Ommatidae, but not in *Micromalthus* and the other groups of Archostemata (Beutel *et al.*, 2008a; Friedrich *et al.*, 2009).
56. Dense vestiture of microtrichia on cuticular surface: (0) absent; (1) present. Present in males of Strepsiptera (Pohl & Beutel, 2005) (Fig. 1G, H).

Adults, head

57. Orientation of head: (0) orthognathous; (1) prognathous or slightly inclined. Orthognathous in Neuroptera and Stylopidia (Fig. 1H), prognathous or slightly inclined in Coleoptera, Megaloptera, Raphidioptera and basal groups of Strepsiptera (Beutel *et al.*, 2008a,b, 2010b, 2011). A variety of head positions has evolved in Polyphaga (e.g. Eucinetidae, Anobiidae, Mordellidae), apparently as results of independent evolution (Lawrence *et al.*, 2011).
58. Constricted neck and postocular extensions: (0) absent or indistinct; (1) present. Strongly constricted neck region and distinct postocular extensions present in Ommatidae and Cupedidae (Beutel *et al.*, 2008a). Constriction also present in *Nicrophorus* Fabricius (Silphidae) (Lawrence *et al.*, 2011).
59. Shape of frontal region: (0) without V- or U-shaped impression; (1) V- or U-shaped impression present. V- or U-shaped impression present on frontal region of males of Strepsiptera excluding †Protoxenidae (Pohl *et al.*, 2005; Pohl & Beutel, 2005).
60. Dorsomedian longitudinal groove on head: (0) absent; (1) present. Present in Hydrophiloidea excluding Spercheidae (Anton & Beutel, 2004; Beutel & Leschen, 2005).
61. Gula: (0) absent; (1) present, membranous or weakly sclerotized and short; (2) present, sclerotized. Present and sclerotized in Coleoptera with very few exceptions (Lawrence *et al.*, 2011). Also present in Raphidioptera and Megaloptera (Röber, 1942; Beutel *et al.*, 2011). Due to the complete fusion of the ventral elements of the head, including the labium, the presence of a gula in Strepsiptera cannot be verified (coded as?).
62. Functional and structural subdivision of compound eyes: (0) absent; (1) present. Morphologically and functionally completely divided in Gyrinidae, with a narrow separating chitinous bar in the groundplan and a broad interocular bridge in Gyrininae (Beutel, 1989a; Beutel *et al.*, 2017). A fully divided compound eye also occurs in the hydrophilid genus *Amphiops* Erichson (Lawrence *et al.*, 2011).
63. Raspberry compound eyes: (0) absent; (1) present. Present in Strepsiptera (Fig. 1G, H), with large ommatidia separated by chitinous bridges densely set with microtrichia (Buschbeck *et al.*, 1999, 2003; Pohl & Beutel, 2005).
64. Ocelli: (0) three well-developed ocelli; (1) two small ocelli; (2) absent. Three well-developed ocelli in Neuropterida excluding Inocellidae (Beutel *et al.*, 2011), two in few groups of Coleoptera [e.g. Hydraenidae partim, Agyrtidae partim (not in *Necrophilus* Latreille), Derodontidae partim, few Leiodidae], and one in some Dermestidae. Absent in most groups of beetles and in Strepsiptera (Leschen & Beutel, 2004; Pohl & Beutel, 2005).
65. M. frontolabralis (M. 8): (0) present, origin on frons; (1) absent. Absent in Coleoptera (e.g. Beutel *et al.*, 2008a,

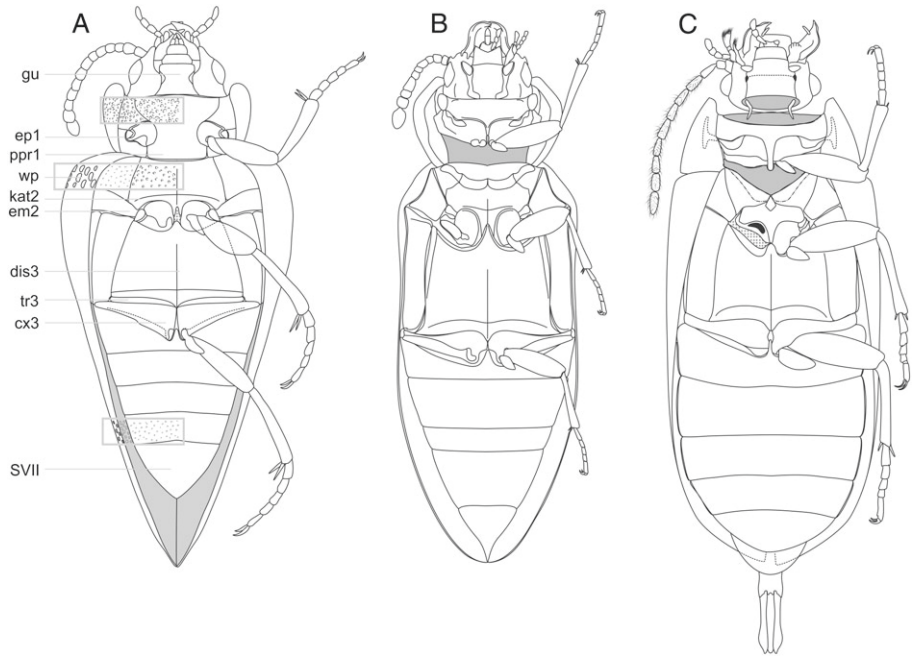

Fig. 2. Hypothesized groundplan conditions and †*Ponomarenkia* (modified from Yan *et al.,* 2017a,b,c). (A) Hypothesized groundplan of Coleoptera s.l. (including stem group): tuberculate surface sculpture; elytrae with window punctures, not closely fitting with abdomen, distinctly projecting beyond abdominal apex; broad prosternal process; procoxal cavities closed, with broad postcoxal bridge; transverse ridge of mesoventrite present; metatrochantin exposed; metacoxae transverse, lacking metacoxal plates; five exposed abdominal ventrites; (B) †*Ponomarenkia* (Coleoptera incertae sedis, †Ponomarenkiidae), close to hypothetical groundplan of crown group of Coleoptera (Yan *et al.*, 2017b): cuticular surface without tubercles or scales; elytra evenly sclerotized, not projecting beyond abdominal apex; propleura exposed; prosternal intercoxal process narrow; procoxal cavities open posteriorly; transverse ridge of mesoventrite present, metatrochantin internalized; (C) Hypothesized groundplan of Polyphaga (Yan *et al.*, 2017a,b): cervical sclerites present; propleura internalized; transverse ridge of mesoventrite absent; metatrochantin internalized; aedeagus trilobed.

2011; Anton *et al.*, 2016, Antunes-Carvalho *et al.*, 2017) and Strepsiptera (Pohl & Beutel, 2005). Present in Neuropterida (Röber, 1942; Achtelig, 1967; Beutel *et al.*, 2011; Zimmermann *et al.*, 2011; Randolf *et al.*, 2013, 2014).

66. Number of antennomeres: (0) > 13; (1) 11; (2) < 11. Multisegmented in Neuropterida (Beutel *et al.*, 2011) and other holometabolous orders. Eleven-segmented in most groups of Coleoptera (e.g. Archostemata, Dytiscoidea, Carabidae, Eucinetidae, Scirtidae, Derodontidae). Less than 11 in Strepsiptera (Fig. 1G, H), Gyrininae, Lepiceridae, Hydroscaphidae, Hydraenidae (partim), Hydrophiloidea, Clambidae, and Scarabaeidae (Anton *et al.*, 2016; Antunes-Carvalho *et al.*, 2017).
67. Antennal flabella: (0) absent; (1) present. Elongate flabella generally present in Strepsiptera (antennomeres 3–7 in the groundplan) (Pohl & Beutel, 2005; Pohl *et al.*, 2005).
68. Hofender's organ: (0) absent; (1) present. Present in males of Strepsiptera as specific sensorial groove of antennomere 4 (Pohl & Beutel, 2005; Pohl *et al.*, 2005).
69. Dense vestiture of dome-shaped antennal chemoreceptors: (0) absent; (1) present. Present in males of Strepsiptera (Pohl & Beutel, 2005; Pohl *et al.*, 2005).
70. Antennal club formed by densely pubescent three distal antennomeres: (0) absent or club formed by different number of segments or without breathing function; (1) present, symmetrical, used as accessory breathing organ; (2) present, strongly asymmetrical. Three-segmented pubescent club present and used as accessory breathing organ in Hydrophiloidea (e.g. Hansen, 1991) (coded as 0 for *Nicrophorus*). Club asymmetrical and mostly three-segmented in Scarabaeoidea (Scholtz & Grebennikov, 2016). Five-segmented club present and used as accessory breathing organ in most groups of Hydraenidae (coded as 0).
71. Pedicellus (0) cylindrical, not ear-shaped; (1) enlarged, ear-shaped, with fringe of long hairs. Highly modified pedicellus functions as receptor of vibrations of the water surface in Gyrinidae (Beutel, 1989a; Beutel *et al.*, 2017).
72. Mandibular mola: (0) absent; (1) present. Absent in Archostemata and Adephaga, and also missing in several groups of Polyphaga [e.g. Staphylinidae (partim), Scirtidae (partim), Eucinetidae (partim), Dascillidae, Scarabaeidae (partim), Elateroidea (partim), Elateroidea] (Lawrence *et al.*, 2011). Present in Myxophaga and in different polyphagan lineages such as Staphylinoidea (major part), Hydrophiloidea s.l. (absent in some histerids), Clambidae, Eucinetidae (major part), Scirtidae (partim), Byrrhidae, Derodontidae, Coccinellidae, Tenebrionidae, and Chrysomelidae (Lawrence *et al.*, 2011).

73. Single movable mandibular preapical tooth (prostheca): (0) absent; (1) present on left mandible. Present on left mandible in Myxophaga (excluding Sphaeriusidae) (Reichardt, 1973; Anton & Beutel, 2006; Lawrence *et al.*, 2011) and Clambidae (Anton *et al.*, 2016). Similar tooth inserted on both mandibles of *Ochthebius* Leach (Hydraenidae) (coded as 0).
74. Galea: (0) present, not palp-like; (1) palp-like; (2) vestigial; (3) fused with lacinia; (4) completely absent. Palp-like and usually composed of two cylindrical smooth galeomeres in Adephaga (Dressler & Beutel, 2010; Lawrence *et al.*, 2011), but one-segmented in Amphizoidae, and one-segmented or absent in Gyrininae (Beutel, 1989a; Beutel *et al.*, 2006, 2017; Lawrence *et al.*, 2011). Vestigial in *Micromalthus* and completely fused with lacinia in Myxophaga. Completely reduced in Strepsiptera excluding †*Protoxenos* Pohl, Beutel and Kinzelbach (Pohl & Beutel, 2005; Pohl *et al.*, 2005).
75. Maxillary palp: (0) composed of several palpomeres; (1) single palpomere. All palpomeres fused in Strepsiptera (Pohl & Beutel, 2005; Pohl *et al.*, 2005).
76. Muscle originating from lateral head capsule and inserting on membrane proximad maxillary base (M. craniobasimaxillaris s. Anton & Beutel, 2012): (0) absent; (1) present. Occurs in Staphyliniformia and Scarabaeoidea (e.g. Anton & Beutel, 2004, 2012; Antunes-Carvalho *et al.*, 2017), but is missing in *Nicrophorus* and *Cetonia* Fabricius (Scarabaeidae).
77. Prementum: (0) present as separate element; (1) absent, completely fused with other labial elements and head capsule. Not present as recognizable separate element in Strepsiptera (Pohl & Beutel, 2005). Labial palps, endite lobes and proximal labial elements also missing.
78. Anterolateral margin of mentum: (0) without rounded lobes; (1) rounded lobes present. Lobes present in Adephaga (e.g. Dressler & Beutel, 2010). Inapplicable in Strepsiptera.
79. Hypopharynx hourglass-shaped between paired mouthparts. Hypopharynx strongly narrowed between paired mouthparts in most groups of Staphyliniformia including Scarabaeoidea (Anton & Beutel, 2004, 2012). Broad in Silphidae and Scydmaeninae (Lawrence, 2016). Inapplicable in Strepsiptera.
80. Dorsal and ventral wall of preoral cavity: (0) with longitudinal epi- and hypopharyngeal bulges with fields of microtrichia; (1) without longitudinal epi- and hypopharyngeal bulges with fields of microtrichia; (2) longitudinal bulges replaced by a antepipharyngeal-prelabial complex. Feeding apparatus with hairy longitudinal epi- and hypopharyngeal bulges present in Myxophaga [e.g. *Lepicerus*, *Satonius* (Endrödy-Younga); Anton & Beutel, 2006] and many groups of Polyphaga (Anton & Beutel, 2004, 2012; Lawrence *et al.*, 2011). Fields of microtrichia on longitudinal bulges interact with brushes on proximal parts of mandibles, probably in correlation with microphagous feeding habits. In all examined species of Elateroidea s.l. and Dascilloidea, longitudinal epi- and hypopharyngeal bulges are replaced by complex of adjacent and densely microtrichious (inframandibular) antepipharynx and prelabium, probably in correlation with extraoral digestion and intake of liquid food (Anton & Beutel, 2012).
81. Salivary glands: (0) present; (1) absent. Absent in Coleoptera and Strepsiptera (Beutel *et al.*, 2011).
82. Salivarium: (0) present; (1) absent. Prelabium and hypopharynx fused in Coleoptera and absent in Strepsiptera as defined separate elements of the head, salivarium thus absent in both groups (Anton & Beutel, 2004, 2012; Beutel & Pohl, 2005; Lawrence *et al.*, 2011). Salivarium generally well developed in adults of Neuropterida (Ferris & Pennebaker, 1939; Achtelig, 1967; Aspöck & Aspöck, 2003; Beutel *et al.*, 2010b; Zimmermann *et al.*, 2011; Randolf *et al.*, 2013, 2014).
83. Mouthfield sclerite: (0) absent; (1) present. Present in Strepsiptera (Kinzelbach; 1971; Pohl & Beutel, 2005).
84. Balloon-gut: (0) absent; (1) present. Large part of digestive tract transformed into air-filled balloon gut in extant Strepsiptera (Beutel & Pohl, 2005; Pohl & Beutel, 2005).

Adults, prothorax

85. Cervical sclerites: (0) present; (1) absent; (2) vestigial. Present in Neuropterida (Ferris & Pennebaker, 1939; Czihak, 1953, 1957; Matsuda, 1956, 1970). Usually absent in Archostemata (Baehr, 1975) (vestigial in *Tetraphalerus* Waterhouse; Friedrich *et al.*, 2009). Always absent in Adephaga (Larsén, 1966; Baehr, 1979) and Myxophaga. Missing in some groups of Polyphaga (e.g. Tenebrionidae, Curculionidae). Distinctly reduced in Chrysomelidae and related families (Larsén, 1966).
86. Lateral connection of pronotum and propleuron: (0) absent; (1) partly or completely connected. Firmly connected in Coleoptera (e.g. Baehr, 1975; Friedrich *et al.*, 2009) and fused in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
87. Protibial apex: (0) without antenna cleaning organ; (1) antenna cleaning organ present. Present in Geadephaga (e.g. Beutel, 1992).
88. Prothoracic trochantin: (0) distinct sclerite; (1) fused with propleura; (2) fused with notum, sternum and pleura; (3) absent. Fused with propleura in Myxophaga and Polyphaga (Hlavac, 1972, 1975). Notum, sternum, pleura, and trochantin completely fused in *Micromalthus* (Lawrence & Newton, 1982). Absent in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
89. Propleura: (0) part of external body wall; (1) concealed, distinctly reduced in size; (2) fused with all other external sclerites of prothorax. Greatly reduced in size and internalized as cryptopleura in Polyphaga (Hlavac, 1972, 1975; Lawrence, 1982). Fused with other external prothoracic sclerites in *Micromalthus* (Lawrence & Newton, 1982) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012)
90. Spinasternum I: (0) well developed; (1) vestigial or absent. Present in Neuropterida (excluding Sialidae; Matsuda,

1970) and Cupedidae (Baehr, 1975; Beutel & Haas, 2000). Absent or strongly reduced in non-archostematan beetles (Doyen, 1966; Baehr, 1975; Friedrich *et al.*, 2009).

91. Apical part of procoxa: (0) without condyle; (1) condyle present. Ventral procoxal condyle present in Adephaga (excluding Gyrininae) (Baehr, 1979; Beutel, 1989b, 1997). Partly reduced in Dytiscidae (coded as 1) (Baehr, 1979).
92. Prothoracic defensive glands: (0) absent; (1) present. Present in Hygrobiidae and Dytiscidae (e.g. Beutel *et al.*, 2006).
93. M. pleuro-occipitalis (M. 7, Itpm1): (0) present; (1) absent. Present in *Chrysopa* (Miller, 1933), *Sialis* (Czihak, 1953), and *Corydalus* Latreille (Kelsey, 1954). Absent in *Agulla* Navás (Matsuda, 1956), *Myrmeleon* (Korn, 1943), and Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2009).
94. M. cervicale-occipitalis torquatus (M. 8, Idvm1?): (0) present; (1) absent. Absent in *Chrysopa* (Miller, 1933), Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2009) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
95. M. mesonoto-postpleuralis (M. 19, IItpm12?): (0) present; (1) absent. Absent in Neuroptera (Miller, 1933; Korn, 1943), Coleoptera (e.g. Larsén, 1966; Baehr, 1975; Friedrich *et al.*, 2009) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
96. M. prospina-mesopleuralis (M. 20, Ispm2): (0) present; (1) absent. Present in Raphidioptera (Matsuda, 1956; Friedrich & Beutel, 2010), Sialidae (Czihak, 1953), and Archostemata (Baehr, 1975; Friedrich *et al.*, 2009). Absent in Neuroptera (Korn, 1943), Corydalidae (Maki, 1936; Kelsey, 1957), non-archostematan Coleoptera (see Friedrich *et al.*, 2009; Friedrich & Beutel, 2010), Strepsiptera, and some other groups, including Hymenoptera (Friedrich & Beutel, 2010).
97. M. profurca-spinalis (M. 21, Ivlm4): (0) present; (1) absent. Present in Raphidioptera (Matsuda, 1956; Friedrich & Beutel, 2010), Megaloptera (except Chauliodinae; Czihak, 1953; Kelsey, 1957), and many other groups (Friedrich & Beutel, 2010). Absent in Neuroptera (Korn, 1943; Friedrich & Beutel, 2010), Coleoptera (Baehr, 1975; Friedrich *et al.*, 2009) and Strepsiptera (Koeth *et al.*, 2012).
98. M. pronoto-coxalis posterior (M. 23, Idvm17): (0) present; (1) absent. Usually present in insects but generally missing in Coleoptera (Friedrich *et al.*, 2009). Whether the pronoto-coxal muscle of Strepsiptera is a homologous muscle is uncertain (Koeth *et al.*, 2012) (coded as?).
99. M. procoxa-cervicalis (M. 26, Ipcm1/2): (0) present; (1) absent. A muscle connecting the procoxa and lateral cervical sclerite is present in Megaloptera (Maki, 1936; Czihak, 1953; Kelsey, 1954), in some groups of Neuroptera (e.g. Osmylidae, Nevrorthidae), and in most other groups of insects. Absent in the myrmeleontiform lineage of Neuroptera (Korn, 1943; Czihak, 1957), in Raphidioptera (Matsuda, 1956), Coleoptera (Friedrich *et al.*, 2009), Strepsiptera (Koeth *et al.*, 2012), and some other groups (Friedrich & Beutel, 2010).
100. M. sterno-coxalis (M. 27, Iscm1): (0) present; (1) absent. Absent in *Myrmeleon* (Korn, 1943), Gyrininae (Larsén, 1966; Beutel, 1989b), Haliplidae, and most groups of Polyphaga (Larsén, 1966; Baehr, 1979; Beutel & Komarek, 2004; Ge *et al.*, 2007).
101. M. pleura-trochanteralis (M. 31, Ipcm8): (0) present; (1) absent. In contrast to Beutel & Haas (2000), present in Megaloptera (Czihak, 1953; Kelsey, 1954) and Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2009). Absent in *Agulla* (Raphidioptera; Matsuda, 1956), Neuroptera (Matsuda, 1970) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
102. M. prospina-mesofurcalis (Ivlm9): (0) present; (1) absent. Present in Neuropterida (e.g. Friedrich & Beutel, 2010), Cupedidae (Baehr, 1975), and many other groups of insects (Friedrich & Beutel, 2008, 2010). Missing in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012), non-archostematan Coleoptera (Larsén, 1966), and in some other groups of insects (e.g. Antliophora; Friedrich & Beutel, 2010).
103. M. profurca-coxalis medialis (Iscm3): (0) present; (1) absent. Present in Neuroptera, Raphidioptera, Sialidae (Korn, 1943; Matsuda, 1956; Friedrich & Beutel, 2010), and most other groups of insects (Friedrich & Beutel, 2008, 2010). Absent in Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2009), Strepsiptera (Koeth *et al.*, 2012) and some other goups of insects (e.g. Trichoptera; Friedrich & Beutel, 2010).
104. M. prospina-coxalis (Iscm5): (0) present; (1) absent. Present in Neuroptera, Sialidae (Czihak, 1953) and many other groups of insects (e.g. Friedrich & Beutel, 2008, 2010). Absent in Raphidioptera (Matsuda, 1956), Corydalidae (Maki, 1936; Kelsey, 1957), Coleoptera (Larsén, 1966; Baehr, 1975; Friedrich *et al.*, 2009), Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012), and Antliophora (Friedrich & Beutel, 2010).

Adults, pterothorax

105. Relative size of pterothoracic segments: (0) almost equally sized; (1) mesothorax distinctly reduced in size. Almost equally sized in most groups of Neuropterida and Mecoptera (e.g. Maki, 1936; Korn, 1943; Czihak, 1953; Matsuda, 1956; Kelsey, 1957; Friedrich & Beutel, 2010). Metathorax strongly enlarged in Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2009) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
106. Relative size of wings: (0) equally sized; (1) hindwings distinctly larger. Modified forewings of Coleoptera and Strepsiptera smaller than hindwings. Hindwings create main or exclusive propulsive force in flight (Kinzelbach, 1971; Friedrich *et al.*, 2009; Koeth *et al.*, 2012). Both pairs of nearly equal size in Neuropterida (e.g. Beutel *et al.*, 2011).
107. Wings of females: (0) present; (1) absent. At least forewings preserved as elytra in females of Coleoptera (with very few exceptions), and usually also membranous

hind wings. Both pairs of wings absent in females of Strepsiptera (Kinzelbach, 1971; Pohl & Beutel, 2005).

108. Connection of meso- and metaventrites: (0) separated; (1) process of metaventrite articulates with mesoventrite; (2) both firmly connected. Distinctly separated from each other in Neuropterida, Strepsiptera and Archostemata (Baehr, 1975; Beutel & Haas, 2000). Metasternal process articulating with posterior mesoventrite in Adephaga (e.g. Beutel, 1992; Beutel & Haas, 2000). Rigidly connected to each other between and within mesocoxal cavities in Myxophaga and Polyphaga (Beutel & Haas, 2000) with few exceptions (Scirtoidea, Derodontidae, Leiodidae partim; e.g. Friedrich & Beutel, 2006; Ge *et al.*, 2007).
109. Katepisternal mesocoxal joint: (0) present; (1) absent. Almost generally present in non-coleopteran holometabolous groups (e.g. Friedrich & Beutel, 2010) and also in Ommatidae and Cupedidae (Baehr, 1975; Friedrich *et al.*, 2009). Absent in Adephaga, Myxophaga, and Polyphaga (Larsén, 1966; Beutel & Haas, 2000).
110. Mesothoracic transverse ridge: (0) present; (1) absent. Present in Neuropterida (e.g. Friedrich & Beutel, 2010), Cupedidae (Baehr, 1975), Ommatidae (Friedrich *et al.*, 2009) and *Sikhotealinia* Lafer (Jurodidae) (Beutel *et al.*, 2008a). Absent in Micromalthidae, Crowsoniellidae and non-archostematan Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2009).
111. Origin of mesofurca: (0) between mesocoxae with common stem; (1) mesofurcal arms separated at base. Origin with common base between mesocoxae in Neuropterida (e.g. Friedrich & Beutel, 2010), Cupedidae and Ommatidae (Baehr, 1975; Friedrich *et al.*, 2009). Base of arms separated in *Micromalthus*, Adephaga (except for some Gyrinidae), Myxophaga, and Polyphaga (e.g. Larsén, 1966; Friedrich & Beutel, 2006).
112. Mesothoracic meron: (0) present; (1) absent (Larsén, 1954). Meron and associated muscles absent in Coleoptera and Strepsiptera (Larsén, 1954; Friedrich & Beutel, 2006; Friedrich *et al.*, 2009; Koeth *et al.*, 2012).
113. Fore wings: (0) unsclerotized; (1) partly sclerotized, reticulate pattern with window punctures; (2) fully sclerotized; (2) transformed into halteres. Transformed into halteres in Strepsiptera (e.g. Kinzelbach, 1971) and into elytra with epipleura in Coleoptera (Lawrence & Newton, 1982). Reticulate pattern with window punctures present in stem-group Coleoptera, Cupedidae and Ommatidae (e.g. Beutel *et al.*, 2008a).
114. Scutellar elytra-locking device: (0) absent; (1) present. Scutellar shield forms triangular elytra-locking device in Coleoptera (Heberdey, 1938).
115. Proximal part of mesocoxae: (0) not recessed into coxal cavities; (1) recessed into cavities. Recessed into cavities in Coleoptera (e.g. Lawrence, 1982).
116. M. mesoscutello-postnotalis (M. 41, IIdlm3): (0) present; (1) absent. Present in Neuropterida and other groups (e.g. Friedrich & Beutel, 2010). Absent in Coleoptera (e.g. Larsén, 1966; Friedrich & Beutel, 2006, 2010), Strepsiptera (Koeth *et al.*, 2012) and most groups of Antliophora (Friedrich & Beutel, 2010).
117. M. mesosterni secundus (M. 43, IIvlm9): (0) present; (1) absent. Absent in *Micromalthus*, in some species of Adephaga, and in Polyphaga with the exception of *Lytta* Fabricius and *Meloe* Linnaeus (Meloidae) (e.g. Larsén, 1966 ; Beutel & Haas, 2000). Not clearly identified in *Tetraphalerus* (Friedrich *et al.*, 2009). Absent in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
118. Mm. mesonoto-sternales (M. 44, IIdvm1): (0) present; (1) absent. Absent in non-archostematan Coleoptera (e.g. Larsén, 1966; Baehr, 1975; Beutel & Haas, 2000; Beutel & Komarek, 2004). Present in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
119. M. mesonoto-pleuralis posterior (M. 46, IItpm6): (0) present; (1) absent. Present in Megaloptera (e.g. Maki, 1936 ; Czihak, 1953 ; Kelsey, 1957), Raphidioptera (Matsuda, 1956; Friedrich & Beutel, 2010), Archostemata (Baehr, 1975; Friedrich *et al.*, 2009), and some groups of Neuroptera (e.g. *Nevrorthus*, *Sisyra* Burmeister; Mickoleit, 1969; Friedrich & Beutel, 2010). Absent in non-archostematan Coleoptera (Larsén, 1966; Beutel & Haas, 2000), Strepsiptera (Koeth *et al.*, 2012), and some other groups including Hymenoptera (Friedrich & Beutel, 2010).
120. M. mesonoto-basalaris (M. 48, IItpm3): (0) present; (1) absent. Present in Neuropterida and most other groups of Holometabola, but absent in Coleoptera (e.g. Larsén, 1966; Friedrich & Beutel, 2006, 2010) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
121. M. mesanepisterno-sternalis (M. 50, IIspm1): (0) present; (1) absent. Present in Neuropterida and almost all other groups of Holometabola (Friedrich & Beutel, 2010). Absent in non-archostematan beetles (e.g. Larsén, 1966; Friedrich *et al.*, 2009) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
122. Mm. mesopleura-alares a and b (M. 53, M. 54, IItpm7, 9): (0) clearly separated; (1) single muscle or two branches inserting on one tendon. With separate origins and insertions in Neuropterida (Maki, 1936; Korn, 1943; Czihak, 1953; Kelsey, 1957), Cupedidae (Baehr, 1975), Ommatidae and *Micromalthus*, but not in non-archostematan Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2009). Muscles absent in Strepsiptera (Koeth *et al.*, 2012).
123. M. intramesanepisternalis (M. 56, IIppm1): (0) present; (1) absent. Absent in *Myrmeleon* (Neuroptera; Korn, 1943), Raphidioptera (Matsuda, 1956), Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2009) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
124. M. mesopleuracosto-praenotalis (M. 57, IItpm2): (0) present; (1) absent. Absent in Coleoptera (e.g. Larsén, 1966; Friedrich *et al.*, 2009) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
125. M. mesonoto-trochantinalis (M. 59, IIdvm2): (0) present; (1) absent. Absent in *Micromalthus* (mesotrochantin not exposed) and *Tetraphalerus* (Friedrich *et al.*, 2009), in some genera of Adephaga, in Myxophaga and Polyphaga

(e.g. Larsén, 1966; Friedrich *et al.*, 2009), and in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).

126. M. mesocoxa-subalaris (M. 64, IIdvm6): (0) present; (1) absent. Absent in Polyphaga (e.g. Larsén, 1966; Beutel & Haas, 2000). Present in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
127. M. mesanepisterno-trochantinalis (M. 68, IIpcm1): (0) present; (1) absent. Absent in *Myrmeleon* (Korn, 1943), Coleoptera (e.g. Larsén, 1966; Baehr, 1975; Friedrich *et al.*, 2009) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
128. M. mesonoto-trochanteralis (M. 69, IIdvm7): (0) present; (1) absent. Absent in *Sphaerius* and all polyphagans examined (e.g. Larsén, 1966; Friedrich & Beutel, 2006) and also in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
129. M. prophragma-mesanepisternalis (IItpm1): (0) present; (1) absent. Present in Neuropterida and other groups (Friedrich & Beutel, 2010). Missing in Coleoptera (Larsén, 1966; Friedrich *et al.*, 2009), Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012) and some other taxa, especially in wingless insects.
130. M. mesofurca-metanepisternalis (M. 88, IIspm6): (0) present; (1) absent. Present in some holometabolan groups including Hymenoptera, Neuroptera (e.g. *Nevrorthus*, Osmylidae), Raphidioptera, Sialidae (Friedrich & Beutel, 2010), and Archostemata (Baehr, 1975; Friedrich *et al.*, 2009). Absent in non-archostematan beetles, in Strepsiptera, and in Antliophora (Friedrich & Beutel, 2010).
131. M. mesonoto-pleuralis medialis (IItpm5): (0) present; (1) absent. Present in Megaloptera (Maki, 1936; Czihak, 1953; Kelsey, 1957), Raphidioptera (Matsuda, 1956), some groups of Neuroptera (Nevrorthidae, Osmylidae), and most other groups of Holometabola (Friedrich & Beutel, 2010). Always absent in Coleoptera (Friedrich *et al.*, 2009) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012), and also in Siphonaptera and other wingless insects (Friedrich & Beutel, 2010).
132. Mesal metacoxal walls: (0) not fused; (1) metacoxae attached to each other along ventromedian edge; (2) mesal walls fused. Fused in adults of Trachypachidae and Dytiscoidea (e.g. Beutel & Roughley, 1988; Beutel, 1997). Connected along ventromesal edges in Gyrininae.
133. Transverse metascutal fissure: (0) absent; (1) present (Brodsky, 1994). Membranous, transverse metascutal fissure (or area) present in Coleoptera (Campau, 1940; Doyen, 1966; Larsén, 1966; Brodsky, 1994; Ge *et al.*, 2007; Friedrich *et al.*, 2009).
134. Metathoracic elytra-locking device: (0) absent; (1) present. Alacristae present and forming locking device in Coleoptera (e.g. Heberdey, 1938; Friedrich *et al.*, 2009).
135. Median division of metapostnotum: (0) absent; (1) present. Present in Neuropterida (Achtelig, 1975).
136. Metacoxae: (0) transverse, recessed into cavities; (1) not transverse, not recessed into cavities. Distinctly transverse in Coleoptera with few exceptions (e.g. Larsén, 1966; Friedrich *et al.*, 2009).
137. Metathoracic trochantin: (0) broad, well developed; (1) reduced, not visible externally; (2) absent. Well developed and visible externally in Neuropterida (Friedrich & Beutel, 2010), Cupedidae and Ommatidae (Baehr, 1975; Friedrich *et al.*, 2009). Absent from external surface in non-archostematan beetles (e.g. Friedrich & Beutel, 2006; Friedrich *et al.*, 2009). Absent in Strepsiptera (Koeth *et al.*, 2012).
138. Number of costal cross veins: (0) < five; (1) > five. More than five in Neuropterida (e.g. Beutel *et al.*, 2011).
139. Hindwing folding: (0) absent; (1) longitudinal and transverse hindwing folding, wings completely covered under elytra in repose. Folded in Coleoptera with very few exceptions (Haas, 1998; Beutel & Haas, 2000; Haas & Beutel, 2001).
140. Apical part of hindwing in resting position: (0) unfolded; (1) rolled; (2) folded. Apical part rolled in Archostemata, *Spanglerogyrus*, Haliplidae, in some small dytiscids, and in few representatives of Polyphaga (e.g. *Artematopus* Perty; Kukalová-Peck & Lawrence, 1993). Folded in other adults of Coleoptera examined (Beutel & Haas, 2000).
141. Oblongum of hindwing: (0) present; (1) absent. Present in Ommatidae, Cupedidae, Myxophaga (with few exceptions), and Adephaga (Beutel & Haas, 2000; Beutel *et al.*, 2008a).
142. Subcubital binding patch: (0) absent; (1) present. Present in *Trachypachus* Motschulsky and Dytiscoidea (excluding Hygrobiidae; Beutel & Roughley, 1988; Beutel & Haas, 2000), and also in some groups of Polyphaga (Heberdey, 1938).
143. Anterior margin of hindwing: (0) not flexible; (1) flexible a bending zone; (2) with a hinge; (3) with a marginal joint. Anterior margin flexible in Coleoptera, but not in outgroup taxa (Haas, 1998; Haas & Beutel, 2001). With a bending zone in almost all groups Polyphaga but with a hinge in the remaining suborders (Kukalová-Peck & Lawrence, 1993, 2004). A marginal joint is present in Scarabaeoidea (Kukalová-Peck & Lawrence, 1993, 2004).
144. Bending zone in medial bar of hindwing: (0) present; (1) absent. Present in Archostemata, Adephaga and Myxophaga (Kukalová-Peck & Lawrence, 1993). Absent in Polyphaga (Kukalová-Peck & Lawrence, 1993) except for Scirtidae and Eucinetidae (Friedrich & Beutel, 2006).
145. Transverse veins of hindwing: (0) present; (1) absent. Generally absent in Strepsiptera with the exception of a single transverse vein in †*Mengea* Grote (Pohl & Beutel, 2005).
146. Shape of wing: (0) elongate, not extended rostrocaudally; (1) fan-shaped, rostrocaudally extended. Fan-shaped in Strepsiptera excluding †Protoxenidae, with a rostrocaudally extended posterior region (Kinzelbach, 1971; Pohl & Beutel, 2005).
147. Distal part of MP_{1+2}: (0) straight or bent anteriorly; (1) bent posteriorly. MP_{1+2} straight or bent anteriorly in non-coleopteran Holometabola, Archostemata, Adephaga

and Hydroscaphidae. Bent posteriorly in all adults of Polyphaga examined (Haas, 1998; Friedrich & Beutel, 2006).

148. Triangular fold: (0) completely absent; (1) RA_{3+4} cut twice by triangular fold; (2) RA_{3+4} not cut twice by triangular fold. Basal portion of RA_{3+4} cut twice by triangular fold in Archostemata, Myxophaga, and Adephaga, but not in Polyphaga (Kukalová-Peck & Lawrence, 1993, 2004).
149. Fulcrum: (0) underneath second axillary; (1) underneath first and second axillary; (2) underneath first axillary. Fulcrum placed underneath second axillary in Archostemata and under first axillary in Myrmeleontidae, Adephaga, Myxophaga, and Polyphaga. Located under both axillary sclerites in Megaloptera and Raphidioptera (Hörnschemeyer, 1998; Friedrich & Beutel, 2006).
150. Second axillary: (0) without a lateral process; (1) process present. Lateral process originating from ventral side of second axillary present in non-archostematan beetles. Absent in Neuropterida and Archostemata (Hörnschemeyer, 1998).
151. Angle between the axis anterior notal process-first axillary and the disto-cranial margin of first axillary: (0) ≥ 50°; (1) <than 45°. Angle of 50° or more in Neuropterida, Archostemata, Adephaga and Myxophaga (Hörnschemeyer, 1998). In Polyphaga 45° or less (Hörnschemeyer, 1998; Friedrich & Beutel, 2006).
152. Shape of metapostnotum: (0) not enlarged and plate-like or shield-like; (1) enlarged and plate-like or shield-like. Enlarged and plate-like in †Protoxenidae (Pohl *et al.*, 2005), very large and shield like in all other strepsipterans (Pohl & Beutel, 2005).
153. M. metascutello-postnotalis (M. 81, IIIdlm3): (0) absent; (1) present. Present in most groups of Holometabola including Neuropterida (Czihak, 1953, 1957; Matsuda, 1956; Kelsey, 1957; Friedrich & Beutel, 2010). Absent in Coleoptera (e.g. Baehr, 1975; Friedrich *et al.*, 2009), Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012) and Antliophora (Friedrich & Beutel, 2010).
154. M. metasterni primus (M. 82, IIvlm3): (0) present; (1) absent. Present in Coleoptera except for *Hydroscapha*, *Sphaerius* and very few polyphagans (e.g. *Cantharis* Linnaeus; Larsén, 1966; Friedrich *et al.*, 2009) and also in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
155. M. metasterni secundus (M. 83, IIvlm5): (0) present; (1) absent. Present in most outgroup taxa (Matsuda, 1970), in Cupedidae (Baehr, 1975), Ommatidae (Friedrich *et al.*, 2009), Trachypachidae (Beutel, 1988), in *Haliplus* Latreille, and in most groups of Carabidae (Larsén, 1966). Absent in Neuroptera (Korn, 1943; Czihak, 1957), Myxophaga, Polyphaga (Friedrich & Beutel, 2006), and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
156. M. metanoto-episternalis brevis (M. 89, IIItpm1): (0) present; (1) absent. Present in Neuroptera (Korn, 1943; Matsuda, 1970), Megaloptera (Maki, 1936; Czihak, 1953; Kelsey, 1957) and Archostemata (Baehr, 1975; Friedrich *et al.*, 2009). Absent in all other groups of Coleoptera (e.g. Larsén, 1966; Beutel & Haas, 2000) and also missing in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
157. M. metanoto-pleuralis medialis (M.91, IIItpm5): (0) present; (1) absent. Present in Neuropterida (except Osmylidae; Czihak, 1953; Matsuda, 1956) and several other holometabolan groups, including Hymenoptera (e.g. Friedrich & Beutel, 2010). Always absent in Coleoptera (see Friedrich *et al.*, 2009) and Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012), and also missing in wingless groups (Friedrich & Beutel, 2008, 2010).
158. M. metanoto-pleuralis b (M. 92, IIItpm6): (0) present; (1) absent. Present in Neuropterida (Maki, 1936; Korn, 1943; Czihak, 1953; Kelsey, 1957; Matsuda, 1970) and Cupedidae (Baehr, 1975; in contrast to Beutel & Haas, 2000). Absent in *Tetraphalerus* and *Micromalthus*, in non-archostematan beetles (e.g. Larsén, 1966; Friedrich *et al.*, 2009), and in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
159. Mm. metapleura-alares a and b (Mm. 95, 96, IIItpm7, 9): (0) separate; (1) with common insertion (Larsén, 1966: M71). Areas of origin and insertion separated in Neuropterida (Maki, 1936; Korn, 1943; Czihak, 1953; Kelsey, 1957; Matsuda, 1970), Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012), and Archostemata (Baehr, 1975; Friedrich *et al.*, 2009). Common insertion on small sclerite proximad third axillary in non-archostematan beetles (e.g. Larsén, 1966).
160. M. metafurca-pleuralis (M. 99, IIIspm2): present; (1) absent. Absent in Coleoptera (Larsén, 1966; Beutel & Haas, 2000). Present in Strepsiptera in contrast to Beutel & Haas (2000) and Koeth *et al.* (2012).
161. M. metanoto-trochantinalis (M. 100, IIIdvm2): (0) present; (1) absent. Absent in *Tetraphalerus*, Adephaga, *Hydroscapha* LeConte, Sphaeriusidae, Hydrophilidae and *Cetonia* Fabricius (e.g. Larsén, 1966; Beutel & Haas, 2000; Friedrich *et al.*, 2009). Also missing in Strepsiptera (Koeth *et al.*, 2012).
162. M. metanoto-coxalis posterior (M. 102, IIIdvm5): (0) present; (1) absent. Absent in *Hydroscapha*, Sphaeriusidae and *Niptus* Boieldieu (Larsén, 1966; Beutel & Haas, 2000), and also in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
163. M. metanepisterno-coxalis (M. 103, IIIpcm4): (0) present; (1) absent. Absent in Adephaga, Elateridae and Scirtoidea (Larsén, 1966; Friedrich & Beutel, 2006), and also missing in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
164. M. metasterno-coxalis (M. 106, IIscm7?): (0) present; (1) absent. Absent in Coleoptera (except for *Ips* De Geer) (Larsén, 1966; Friedrich *et al.*, 2009) and also missing in Strepsiptera (Kinzelbach, 1971; Koeth *et al.*, 2012).
165. M. metafurca-coxalis posterior (M. 109, IIIscm2): (0) present; (1) absent. Present in Neuropterida (Friedrich & Beutel, 2010) and all beetles examined with the exception of Dytiscoidea (Larsén, 1966; Beutel & Haas, 2000) and Scirtoidea (Friedrich & Beutel, 2006). Absent in Strepsiptera (Koeth *et al.*, 2012).

166. M. metanepisterno-trochantinalis (M. 110, IIIpcm1): (0) present; (1) absent. Absent in Coleoptera (Larsén, 1966; Beutel & Haas, 2000; Friedrich *et al.*, 2009), Strepsiptera (Koeth *et al.*, 2012), and Neuroptera (Korn, 1943).
167. M. metatrochantero-basalaris (M. 112, IIIpcm5): (0) present; (1) absent. Present in Neuropterida (Friedrich & Beutel, 2010). Absent in all non-archostematan beetles and *Tetraphalerus* (Larsén, 1966; Friedrich *et al.*, 2009). Also absent in Strepsiptera (Koeth *et al.*, 2012).
168. Muscle between metafurcal arm and first abdominal stigma: (0) present; (1) absent. Present in Neuropterida but absent in Coloptera and Strepsiptera (Larsén, 1966; Friedrich *et al.*, 2009; Koeth *et al.*, 2012). Also missing in other holometabolous groups (Achtelig, 1975; Friedrich & Beutel, 2010).

Adults, abdomen

169. Transverse suture of abdominal tergite I: (0) absent; (1) present. Present in Neuropterida (Achtelig, 1975).
170. Abdominal sternite I: (0) strongly reduced or absent, not visible externally; (1) present, exposed. Reduced in Coleoptera (Lawrence, 1982; Beutel, 1997) (Fig. 2). Partly integrated into metathorax in Strepsiptera (Kinzelbach, 1971; coded as 0).
171. Transverse suture of abdominal sternite II: (0) absent; (1) present. Present in Neuropterida (Achtelig, 1975).
172. Division of abdominal sternite II: (0) not divided by hind coxae; (1) completely divided. Completely divided in Adephaga (Lawrence *et al.*, 2011).
173. Median ridge of sternite II: (0) absent; (1) present. Present in Cupedidae and Ommatidae (Beutel *et al.*, 2008a).
174. Abdominal segments IX and X: (0) exposed; (1) retracted into abdominal apex. Concealed within the preceding abdominal segments in Coleoptera (e.g. Lawrence, 1982) (Fig. 2).
175. Configuration of segment IX: (0) not ring-shaped and strongly sclerotized; (1) ring-shaped and strongly sclerotized. Ring-shaped and strongly sclerotized in males of Strepsiptera (Pohl & Beutel, 2005, 2008).
176. Shape of male tergite X: (0) not narrowed and elongated; (1) narrowed and elongated. Narrow and elongated in males of Strepsiptera (Pohl & Beutel, 2005, 2008).
177. Genital appendages IX of females: (0) separate; (1) fused, with intrinsic muscles; (2) absent. Fused and equipped with intrinsic muscles in females of Neuropterida (Mickoleit, 1973). Absent in Strepsiptera (Kinzelbach, 1971; Pohl & Beutel, 2005, 2008).
178. Trichobothria field on tergum X: (0) absent; (1) present. Present in Neuropterida (Aspöck & Aspöck, 2008).
179. Arrangement of trichobothria field on tergum X: (0) band-shaped; (1) rosette-shaped. Rosette-shaped in Neuroptera and Megaloptera (Aspöck & Aspöck, 2008).
180. Eversible sacs of segment XI: (0) absent; (1) present. Present in both subgroups of Megaloptera, assigned to fused gonocoxites XI by Aspöck & Aspöck (2008).
181. Number of Malpighian tubules in adults: (0) eight; (1) six; (2) four; (3) vestigial or absent. Eight in Neuroptera (excluding Coniopterygidae) and, six in Raphidioptera, Sialinae (Aspöck & Aspöck, 2003), Myxophaga, Hydrophiloidea, Eucinetoidea, Derodontidae, and some other groups of Polyphaga (Lawrence, 1982). Four in Mengenillidae (females), Archostemata, Adephaga, and many groups of Polyphaga (Lawrence, 1982). Usually reduced in Strepsiptera. Vestigial but recognizable in males *Xenos* (Kinzelbach, 1971; Pohl & Beutel, 2005, 2008).
182. Condition of Malpighian tubules in adults: (0) free; (1) cryptonephric. Cryptonephric in Cucujiformia (Lawrence, 1982; Lawrence *et al.*, 2011) and terrestrial larvae of Neuroptera (Aspöck & Aspöck, 2003; coded as 0 for neuropteran terminals). Coded as inapplicable for Strepsiptera.

Characters related to reproduction and development

183. Torsion of aedeagus: (0) absent; (1) present. Rotated during repose (90°) and during copulation (180°) in Adephaga (excluding Gyrinidae) (Beutel & Roughley, 1988). Also everted asymmetrically in Scydmaeninae and other subgroups of Staphylinidae (Beutel & Leschen, 2005; Lawrence *et al.*, 2011; Newton, 2016).
184. Configuration of aedeagus: (0) composed of different elements, not simple and blade-like; (1) simple and blade-like. Aedeagus usually composed of basal piece, parameres and median lobe in beetles (Lawrence, 1982; Beutel, 1997). Also, structurally complex in Neuropterida (Aspöck & Aspöck, 2008). Simple and blade-like in Strepsiptera (Kinzelbach, 1971; Pohl & Beutel, 2005). Male genitalia highly variable in *Zorotypus* (coded as?).
185. Ovarioles: (0) developed; (1) reduced. Completely reduced in females of Strepsiptera, eggs float in body cavity (Kinzelbach, 1971; Pohl & Beutel, 2005, 2008).
186. Birth organs: (0) absent; (1) present. Present in females of Strepsiptera (Kinzelbach, 1971; Pohl & Beutel, 2005, 2008).
187. Mode of copulation: (0) not traumatic; (1) traumatic. Traumatic insemination or penetration in Strepsiptera (Silvestri, 1941; Peinert *et al.*, 2016).
188. Mode of reproduction: (0) oviparous; (1) viviparous. Viviparous in Strepsiptera (Kinzelbach, 1971; Kathirithamby, 1989; Pohl & Beutel, 2005, 2008).
189. Egg deposition: (0) laid without cocoon or egg case; (1) one side of single egg covered by web; (2) eggs enclosed in silk cocoons or egg case. One side of single egg covered by web in Hydraenidae, eggs enclosed in silk cocoon or egg case in Hydrophiloidea (Hansen, 1991, 1997). Coded as inapplicable for Strepsiptera.
190. Puparium: (0) absent; (1) present. Formed by exuvia of last secondary larva in Strepsiptera (e.g. Kinzelbach, 1971; Pohl & Beutel, 2005, 2008) (Fig. 1D).

Results

The parsimony analysis of the complete matrix (190 characters) with TNT yielded 12 minimum length trees (83 with NONA) of 402 steps length, with a pattern Strepsiptera + (Archostemata + (Adephaga + (Myxophaga + Polyphaga))) (Fig. 4: strict consensus tree). The monophyly of Coleopterida is very strongly supported [branch support (bs) = 15] whereas it is quite low for Coleoptera (bs = 2) and also for Coleoptera excluding Archostemata (bs = 2). The bs is 6 for Adephaga, 3 for Myxophaga + Polyphaga, 4 for Myxophaga, and 5 for Polyphaga.

The Bayesian analysis (Figures S1 and S2) yielded a similar pattern, but with the noteworthy exception that the strepsipteran terminals are placed as sister group of Coleoptera excluding Archostemata [posterior probability (pp) = 0.769]. The pp for Coleopterida and Strepsiptera was 1. In parsimony analyses a pattern (Polyphaga (+ Adephaga + (Myxophaga + Archostemata))) (McKenna *et al.*, 2015) required nine additional steps (tree length 411) and (Polyphaga (+ Archostemata + (Adephaga + Myxophaga))) (Kukalová-Peck & Lawrence, 1993, 2004) 11 (tree length 413).

Unambiguous apomorphies of major clades obtained in parsimony analysis (homoplasious changes in italics) (see Fig. *4*).

Coleopterida (bs = 17)

14.1, *M. frontolabralis absent in larvae* (present in first instars of *Tenomerga*); 34.2, salivary ducts and glands absent; 64.2, ocelli absent (with few reversals in Coleoptera); 65.1, M. frontolabralis absent; 81.1, salivary glands absent; 82.1, salivarium absent; 85.1, *cervical sclerites absent* (vestigial in Ommatidae and present in many groups of Polyphaga); 86.1, pronotum and propleuron laterally connected; 94.1, M. cervicale-occipitalis torquatus absent; 102.1, M. prospina-mesofurcalis absent (implies reversal in Archostemata); 103.1, M. profurca-coxalis medialis (Iscm 3) absent; 112.1, mesocoxal meron absent; 116.1, M. mesoscutello-postnotalis (M. 41, IIdlm3) absent; 120.1, M. mesonoto-basalaris (M. 48, IItpm3) absent; 125.1, M. mesonoto-trochantinalis (M. 59, IIdvm2) absent; 129.1, M. prophragma-mesanepisternalis (IItpm1) absent; 161.1, M. metanoto-trochantinalis (M. 100, IIIdvm2) absent; 164.1, M. metasterno-coxalis (M. 106, IIscm7?) absent; 170.0, abdominal sternite I strongly reduced or absent; 181.2, *four Malpighian tubules* (?).

Strepsiptera (bs = 48, pp 1)

1.1, length of first instars < 0.5 mm; 3.1, tentorium of larvae reduced; 6.2, head of endoparasitic secondary larvae rounded and simplified; 12.2, larval labrum completely fused with head capsule; 13.1, anterior margin of head forms sharp cutting edge; 15.1, M. frontoepipharyngalis absent; 16.1, antenna of primary larva extremely reduced; 19.2, mandibles largely internalized, ventral mouthparts partly fused with head capsule; 24.3, maxillae medially connected, plate-like; 27.2, galea absent in primary larvae; 29.1, maxillary palp present as bolt-shaped socket and seta; 33.1, double opening of preoral cavity; 35.1, brain shifted to middle body region; 37.1, sternal plates present; 39.2, five leg segments, femur fused with trochanter; 40.2, lobe-like pretarsal attachment pads; 42.1, posterior edges of abdominal sternites with bristles and spinuale; 50.1, terminal abdominal jumping apparatus of first instars present; 52.1, larvae endoparasitic; 56.1, cuticle of males with dense vestiture of microtrichia; 59.1, frontal region with V- or U-shaped impression; 63.1, raspberry compound eyes; 67.1, antennal flabella present; 68.1, Hofeneder's organ present; 69.1, dense vestiture of antennal dome-shaped chemoreceptors; 75.1, maxillary palp one-segmented; 77.1, prementum fused with other labial parts and head capsule; 83.1, mouthfield sclerite; 84.1, balloon gut; 88.2, protrochantin absent; 89.2, *propleura fused with all other external prothoracic sclerites*; 107.1, females completely wingless; 117.1, *M. mesosterni secundus (M. 43*, *IIvlm9)* of males *absent*; 128.1, *M. noto-trochanteralis mesothoracis* (*M. 69*, *IIdvm7*) *absent*; 137.2, metatrochantin reduced, not visible externally; 145.1, transverse veins of hindwings absent; 146.1, hindwings fan-shaped; 152.1, metapostnotum enlarged, plate-like or shield-like; 162.1, *M. noto-coxalis posterior metathoracis (M. 102*, *IIIdvm5) absent*; 165.1, *M. furca-coxalis posterior metathoracis (M. 109*, *IIIscm2) absent*; 175.1, segment IX of males ring-shaped and fully sclerotized; 176.1, tergite X of males elongated and narrow; 177.2, female genital appendages IX absent; 184.1, aedeagus formed of single element and blade-like.

Coleoptera (bs = 1)

53.1, pupal mandible immobilized; 54.1, sclerites firmly connected, no membranes exposed; 114.1, scutellar elytra-locking device present; 115.1, proximal part of mesocoxae recessed into cavities; 133.1, transverse metascutal fissure present; 134.1, alacristae forming metascutal elytral locking device; 139.1, longitudinal and transverse hindwing folding; 141.0, oblongum of hindwing present; 143.1, anterior margin of hindwing with a hinge; 160.1, M. metafurca-pleuralis (M. 99, IIIspm2) absent; 174.1, abdominal segments IX and X retracted into abdominal apex.

Coleoptera excluding Archostemata (bs = 1)

118.1, Mm. mesonoto-sternales (M. 44, IIdvm1) absent; 122.1, Mm. mesopleura-alares a and b (M. 53, M. 54, IItpm7, 9) present as single muscle or two branches inserting on one tendon; 137.1, metatrochantin absent from surface; 150.1, lateral process of second axillary present; 159.1, Mm. metapleura-alares a and b (Mm. 95, 96, IIItpm7, 9) with common insertion.

Polyphaga + Myxophaga (bs = 3)

5.0, *larval head subprognathous*; 17.3, three larval antennomeres; 38.1, *larval leg with single claw*; 39.1, *larval leg with tibia and tarsus fused*; 72.1, *mandible of adults with mola*; 80.1, *dorsal and ventral wall of preoral cavity with longitudinal epi-*

and hypopharyngeal bulges with fields of microtrichia (modified or absent in many groups of Polyphaga); 88.1, protrochantin fused with propleura.

Polyphaga (bs = 4)

85.0, *cervical sclerites well developed* (absent in several groups); 89.1, propleura internalized; 100.1, *M. sterno-coxalis (M. 27*, *Iscm1) absent* (also absent in some adephagans); 117.1, *M. mesosterni secundus (M. 43*, *Ilvlm9) absent* (occurring in Meloidae); 126.1, M. mesocoxa-subalaris (M. 64, IIdvm6) absent; 141.1, *oblongum of hindwing absent*; 147.1, anterior margin of hindwing with a bending zone; 148.2, *bending zone in medial bar of hindwing absent*; 151.1, angle between the axis anterior notal process and the disto-cranial margin of first axillary less than 45°.

For autapomorphies of Archostemata, Myxophaga and Adephaga, see Beutel (1993, 1997), Beutel & Hörnschemeyer (2002a,b), Beutel *et al.* (1999, 2006), and Lawrence *et al.* (2011).

Discussion

Monophyly of Coleopterida

The morphological support for a clade comprising Strepsiptera and Coleoptera was considered weak or moderate in earlier studies (Friedrich & Beutel, 2010; Beutel *et al.*, 2011). A surprising result in the present contribution was a very strong branch support value for this unit (bs 17, pp: 1) in the parsimony analysis and numerous potential synapomorphies, only some of them linked with posteromotorism. Shared derived features of the adult head are the reduced number of antennomeres, the loss of M. frontolabralis, and the absence of the salivarium, salivary ducts and salivary glands. Another potential synapomorphy is the far-reaching or complete reduction of the ocelli, which are always absent in strepsipterans and present as small vestiges in a few groups of Coleoptera, arguably due to reversal (Leschen & Beutel, 2004; Beutel *et al.*, 2008a). Derived features of the prothorax are the lateral connection of the pronotum and propleuron and the loss of four muscles, two of them associated with the prospina and one belonging to the neck region. The loss of the mesocoxal meron and the separation of the mesofurcal arms are derived features of the mesothorax. Several muscle losses are probably linked to posteromotorism (not coded as a single character here), with a distinctly shortened mesothorax with halteres or elytra, and an enlarged metathorax with the functional wings (Figs 1–3). This clearly distinguishes Coleopterida from all other groups of Holometabola, which are characterized either by similarly sized pterothoracic segments and wing pairs (Neuropterida, Mecoptera) or by anteromotorism, either with functional (Hymenoptera) or anatomical (Diptera) dipterism (Beutel *et al.*, 2011; Peters *et al.*, 2014). Characters of the abdomen play a minor role, if at all. The reduced first abdominal sternite is apparently linked to the enlargement of the metathorax and metacoxae. The reduced number of four Malpighian tubules is probably due to convergence, as six are present in many beetles (Lawrence, 1982). Even though the minute first instars of Strepsiptera appear quite similar to larvae of some groups of Coleoptera, notably in the predacious Adephaga, our analyses did not yield any synapomorphies of the immature stages. A large and distinctly sclerotized pronotum is arguably a shared derived feature, even though this varies strongly in Coleoptera and more or less distinctly defined pronota also occur in other groups, especially in Neuropterida (Beutel *et al.*, 2010a). Prognathism and the fused labrum are features linked with carnivorous habits in some groups of Coleoptera (e.g. Adephaga, Hydrophiloidea, Elateroidea) (Beutel, 1993, 1995, 1999), but with endoparasitism in the case of Strepsiptera (Kinzelbach, 1971; Pohl & Beutel, 2008, 2013), i.e. with the necessity to penetrate the host's body wall.

A surprising result of the Bayesian analysis of the morphological data was the inclusion of Strepsiptera in Coleoptera, thus rendering the latter paraphyletic (Figures S1 and S2). The placement of the strepsipteran terminal as sister group of Myxophaga + Polyphaga is in contrast to earlier hypotheses with 'Stylopidae' as a specialized family of cucujiform Polyphaga (Crowson, 1981), and also to the recent evaluations of genomes (Niehuis *et al.*, 2012) and transcriptomes (Boussau *et al.*, 2014; Misof *et al.*, 2014; Peters *et al.*, 2014) supporting Coleoptera as a clade. Numerous autapomorphies of Strepsiptera suggest long branch attraction (LBA) as a possible reason for the apparent artifact. However, likelihood analyses (including Bayesian inference) are considered less sensitive to LBA than parsimony (Bergsten, 2005; Kjer *et al.*, 2016). Moreover, Strepsiptera were even more deeply nested in Coleoptera (sister to Myxophaga + Polyphaga) after the exclusion of 36 presumptive autapomorphies of Strepsiptera. It is conceivable that establishing a suitable model for the data is a problem in Bayesian analyses of morphological characters. On principle, it cannot be fully excluded that Strepsiptera are nested in Coleoptera (McKenna & Farrell, 2010). However, this would not only be in clear contrast to recent analyses of genomic and transcriptomic data (Niehuis *et al.*, 2012; Boussau *et al.*, 2014; Misof *et al.*, 2014; Peters *et al.*, 2014), but would also imply unlikely transformations of morphological characters, such as the formation of gyroscopic sense organs from elytra, extensive secondarily exposed membranes, and secondarily everted and very specifically modified terminal abdominal segments.

Numerous apomorphies of Strepsiptera are distributed among all life stages and both sexes (e.g. Pohl & Beutel, 2005, 2008) (Fig.1). Some of them are linked to the exceptional flight performance of the adult males (Fig. 1G, H), for instance the dense vestiture of microtrichia on the body surface or the unusually large dorsal longitudinal muscles in the metathorax (Fig. 3C). However, most of them are more or less closely related to endoparasitism, as, for instance, the attachment structures and reduced antennae of the minute primary larvae, the far-reaching simplification of the secondary larvae (not coded here as a single character) (Fig. 1A-D), the birth organs and reduced ovaries of females, vivipary and traumatic insemination or penetration (Silvestri, 1941; Peinert *et al.*, 2016), and the raspberry compound eyes (KInzelbach, 1971; Buschbeck *et al.*, 1999, 2003) and

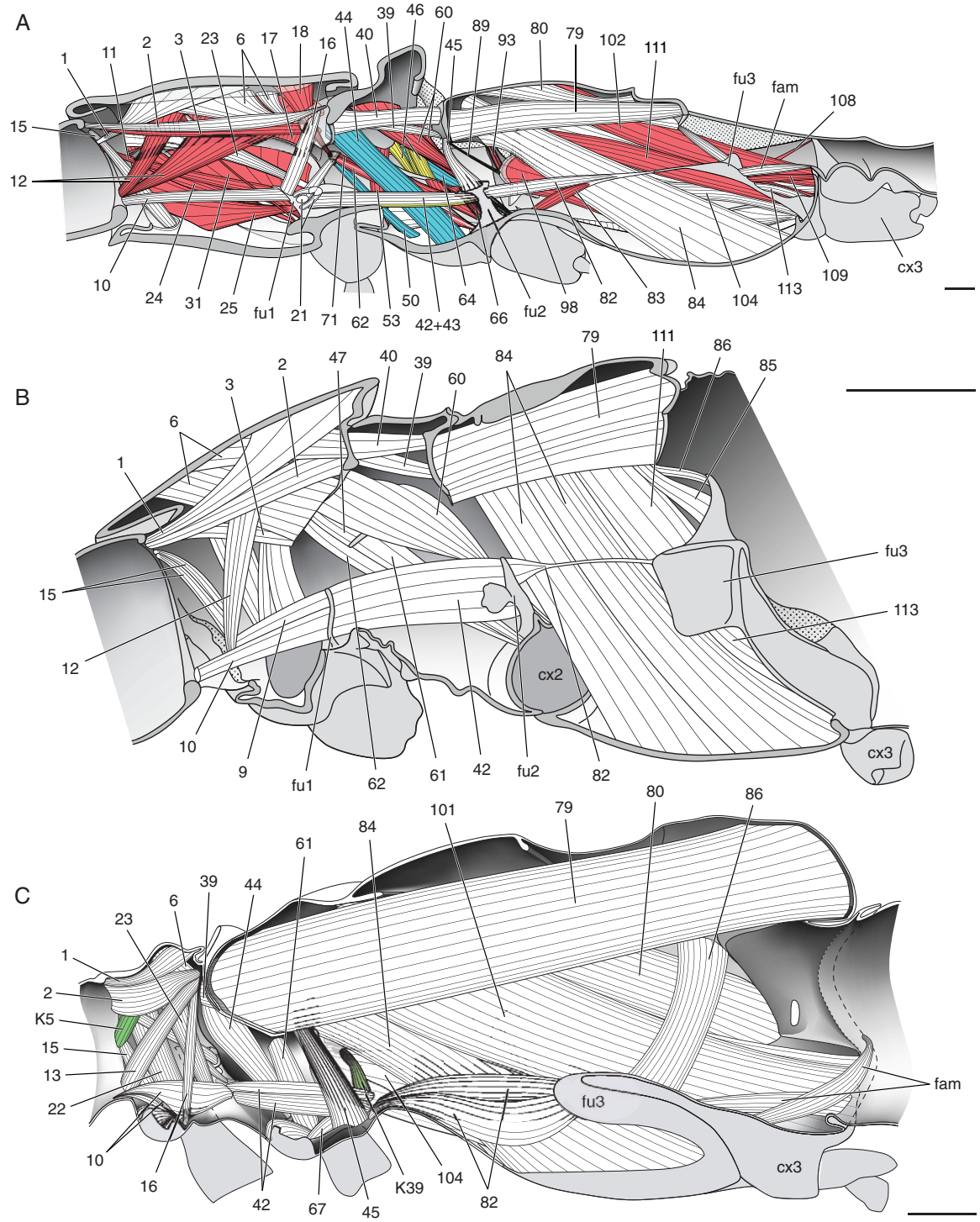

Fig. 3. Comparison of the thoracic muscular system of representatives of: (A) Archostemata (*Tetraphalarus*, Ommatidae; modified from Friedrich *et al.*, 2009); (B) Polyphaga (*Helophorus*, Helophoridae; redrawn from Beutel & Komarek, 2004); and (C) Strepsiptera (*Mengenilla*, Mengenillidae; modified from Koeth *et al.*, 2012). Muscle numbers are based on Beutel & Haas (2000) and Friedrich *et al.* (2009). Muscles present in Archostemata (plesiomorphies), but absent Polyphaga and Strepsiptera (convergent loss) are coloured in yellow. Muscles in red and blue are lacking in Strepsiptera or Polyphaga, respectively. Muscles in green in *Mengenilla* are absent in Coleoptera, but are probably groundplan features of Coleopterida. Note that muscle losses are largely restricted to the mesothorax in Coleoptera, whereas a similar degree of reduction affects all thoracic segments in strepsipterans. [Colour figure can be viewed at wileyonlinelibrary.com].

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sperm pump of the short-lived males (Kinzelbach, 1971; Pohl & Beutel, 2005, 2008).

It is noteworthy that the inclusion of strepsipteran terminal taxa in this study (in contrast to Beutel & Haas, 2000 and Friedrich *et al.*, 2009) and additional character sets (mainly from Friedrich & Beutel, 2010 and Beutel *et al.,* 2011) resulted in distinctly reduced branch support for Coleoptera (Fig. 4), 1 compared with > 20 in Beutel & Haas (2000) and Friedrich *et al.* (2009). Features previously interpreted as groundplan apomorphies of beetles probably evolved earlier in the stem group of Coleopterida. This includes posteromotorism as the most conspicuous character complex but also features other body parts as outlined earlier. Despite the comparatively low branch support value, Coleoptera are still clearly supported by ten autapomorphic character states, especially features frequently associated with a strongly armoured body, including the absence of external membranes, the formation of elytra, and invaginated terminal segments.

The coleopteran subordinal relationships

The phylogeny presented here (Fig. 4) is compatible with the results of earlier cladistic studies based on morphological data (Beutel & Haas, 2000; Beutel *et al.*, 2008a; Friedrich *et al.*, 2009). Archostemata are placed as sister group of the remaining three suborders, and Myxophaga as sister group of Polyphaga (also in Bayesian analyses with Coleoptera enforced as monophyletic: Figure S3). It is noteworthy that the present extended dataset, as in the case of the entire Coleoptera, reduces the support value of this possible clade compared with previous analyses (Beutel & Haas, 2000; Friedrich *et al.*, 2009): 1 versus 5 or 11, respectively. The potential synapomorphies of Coleoptera excluding Archostemata were already discussed in earlier studies (Beutel & Haas, 2000; Beutel *et al.*, 2008a; Friedrich *et al.*, 2009). That Archostemata (especially Cupedidae and Ommatidae) are the coleopteran subgroup with a maximum of preserved plesiomorphies is largely undisputed (Lawrence, 1999; Beutel & Haas, 2000). This includes the cuticular pattern with tubercles and scales, which is very similar to the cuticular surface of Permian stem group coleopterans (e.g. †Tshekardocoleidae, †Permocupedidae; Ponomarenko, 1969; Beutel *et al.*, 2008), elytra with rows of unsclerotized window punctures, the presence of a transverse ridge on the mesoventrite, loosely connected meso- and metaventrites, an exposed metatrochantin, and a pterothoracic muscle set distinctly more complex than in the other suborders, especially Myxophaga and Polyphaga (Beutel & Haas, 2000).

Similar to previous analyses based on morphology, but in contrast to Kukalová-Peck & Lawrence (1993, 2004) and molecular studies (e.g. Misof *et al.*, 2014; Peters *et al.*, 2014; McKenna *et al.*, 2015), the small order Myxophaga is placed as sister group of the megadiverse Polyphaga. This is suggested by several shared derived features of larvae and adults, in the former the fusion of the tibia and tarsus and a single claw, and in the latter the fusion of the protrochantin and propleura (Hlavac, 1972, 1975; Lawrence, 1982; Beutel, 1997; Beutel & Haas, 2000). Additionally, the meso- and metaventrite are firmly connected in all myxophagans and almost all groups of Polyphaga, with the notable exception of Scirtoidea (and a few members of Leiodidae) (e.g. Beutel & Haas, 2000; Friedrich & Beutel, 2006; Ge *et al.*, 2007).

An important character and potential synapomorphy of Polyphaga and Myxophaga is a complex feeding apparatus with epi- and hypopharyngeal bulges with fields of microtrichia interacting with mandibular brushes (Anton & Beutel, 2004, 2012; Anton *et al.*, 2016; Antunes-Carvalho *et al.*, 2017). This suggests feeding on decaying materials (saprophagy) or small particles (e.g. fungal spores) as a groundplan feature of both suborders. It is conceivable that this preoral configuration was secondarily lost in the other two suborders, in the case of Adephaga in correlation with carnivorous habits and preoral digestion, and in the case of Archostemata linked with limited food uptake of the adults. In this case, the presence of the preoral apparatus would be a complex derived groundplan feature of Coleoptera.

An alternative phylogenetic scenario is the placement of Polyphaga as sister group of the remaining three suborders. This was suggested based on characters of the hindwing (Kukalová-Peck & Lawrence, 1993, 2004), in recent molecular studies based either on transcriptomes (Misof *et al.*, 2014; Peters *et al.*, 2014) or analyses of eight single genes (Wiegmann *et al.*, 2009), and also by unpublished results of analyses of transcriptomes with a strongly extended taxon sampling (*c*. 130 terminals including strepsipterans and neuropterids). Using our morphological dataset, this requires considerably more evolutionary steps, 411 (instead of 402) with either Adephaga (Misof *et al.*, 2014) or Myxophaga (McKenna *et al.*, 2015) as sister taxon of Archostemata, and 413 with Archostemata as sister group of Adephaga + Myxophaga, as suggested by Kukalová-Peck & Lawrence (1993, 2004).

The evolutionary scenario differs strongly with Polyphaga as sister group of the remaining suborders. It is in fact the most derived subgroup in terms of thoracic structural features, including a series of muscle reductions (Beutel & Haas, 2000; Friedrich *et al.*, 2009). An enforced topology with a clade Adephaga + Myxophaga + Archostemata yields three potential synapomorphies for this unit, the absence of cervical sclerites (85.1; also missing in Strepsiptera, vestigial cervical sclerites present in Ommatidae), the absence of M. mesonoto-trochanteralis (M. 69) (128.0; also missing in Strepsiptera, present in outgroup taxa), hindwings with a marginal joint (143.2), the presence of a bending zone in the medial bar of the hindwing (144.1; also present in Scirtoidea), and RA3 + 4 cut twice by a triangular fold (148.1). Among these arguments, most of them already suggested by Kukalová-Peck & Lawrence (1993, 2004), the marginal joint (Haas, 1998; Haas & Beutel, 2001) appears as a convincing evolutionary novelty, suggesting a simple flexible anterior hindwing margin as a groundplan feature of Coleoptera. The reconstruction of character evolution under this scenario (Mesquite; Maddison & Maddison, 2011) suggests numerous character reversals in Archostemata, especially concerning the pterothorax. This would include a secondarily acquired transverse ridge of the mesoventrite, the

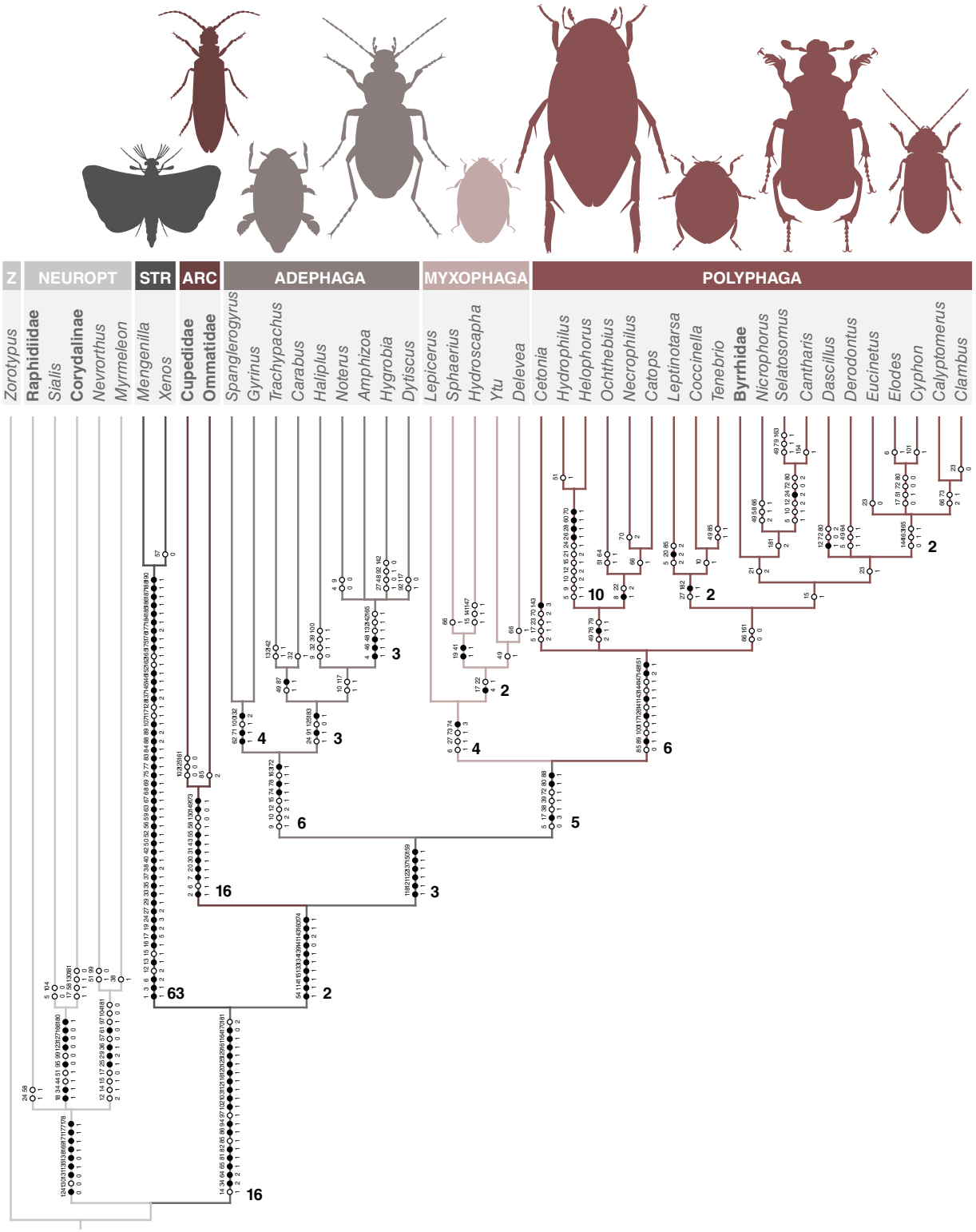

Fig. 4. Phylogeny based on parsimony analysis of 190 morphological characters. Strict consensus of 12 trees (402 steps, CI: 058) obtained with TNT. Unambiguous apomorphies mapped on branches (filled circles nonhomoplasious). Branch support values (see list of apomorphies). [Colour figure can be viewed at wileyonlinelibrary.com]

reappearance of elytral window punctures and cuticular tubercles and scales, a secondarily exposed metatrochantin, and the reacquisition of an entire series of thoracic muscles (e.g. M. noto-sternalis mesothoracis, M. metasterni primus, M. metanotrochantinalis), which are present in the neuropterid outgroup taxa. This interpretation appears less likely than a numerically less parsimonious alternative scenario: preserved plesiomorphic traits in the wood-associated Archostemata, and multiple parallel character transformations in the other suborders. Presumably ancestral archostematan features were lost not only in the three extant suborders Polyphaga, Adephaga and Myxophaga, but also in extinct groups assigned to Archostemata such as †Schizophoridae, †Catiniidae or †Ademosynidae (Ponomarenko, 1969; Beutel *et al.*, 2008a). A strong and uniform selective pressure was probably linked to the switch from ancestral wood-associated habits (e.g. preference for subcortical spaces) to alternative lifestyles, e.g. in riparian or aquatic habitats. This is a possible explanation for the independent evolution of similar morphological syndromes in Myxophaga and the two large extant suborders.

A clade comprising Myxophaga and Polyphaga is not supported in recently published molecular phylogenies (e.g. Bocak *et al.*, 2014; Misof *et al.*, 2014; McKenna *et al.*, 2015). Closer affinities of the latter with Archostemata (McKenna *et al.*, 2015) or Archostemata + Adephaga (Misof *et al.*, 2014) imply that presumptive synapomorphies like the lack of a separate protrochantin and a five-segmented larval leg and single larval claws (Lawrence, 1982; Beutel & Haas, 2000) must have evolved independently. The similar feeding apparatus of basal polyphagan lineages (Anton & Beutel, 2004, 2012; Anton *et al.*, 2016; Antunes-Carvalho *et al.*, 2017) and Myxophaga (Anton & Beutel, 2006; Yavorskaya *et al.*, 2018) is probably ancestral for Coleoptera as pointed out earlier, and apparently linked to primarily saprophagous feeding habits.

Relationships within the suborders

The taxon sampling of Archostemata, which includes only two families mostly characterized by plesiomorphic features (Ommatidae, Cupedidae) (Beutel *et al.*, 2008a; Friedrich *et al.*, 2009), does not allow any conclusions on the intrasubordinal relationships.

The well-supported Adephaga display a mixture of specializations associated with predacious habits (e.g. extraoral digestion in larvae and adults) with a relatively unspecialized condition of thoracic sclerites, with an exposed propleuron, free protrochantin, articulated meso- and metaventrites, and a relatively complete pterothoracic muscle set (Larsén, 1966; Beutel & Haas, 2000). As in earlier contributions based on morphology (Beutel & Roughley, 1988; Beutel *et al.*, 2006, 2013), the specialized surface-swimming Gyrinidae are placed as sister group of the remaining adephagan families. They were included in monophyletic Hydradephaga in some molecular studies (Shull *et al.*, 2001; McKenna *et al.*, 2015). However, a basal position was also retrieved in a recent analysis of ultraconserved elements (UCEs) (Baca *et al.*, 2017) and in recent analyses of transcriptomes (D. McKenna, unpublished data). As in earlier morphology-based analyses (Beutel & Roughley, 1988; Beutel *et al.*, 2013). Haliplidae were placed as sister group of Dytiscoidea, and Geadephaga were retrieved as a monophyletic unit. This is also in agreement with results of Baca *et al.* (2017) based on UCEs and with recent transcriptome analyses (D. McKenna, unpublished data).

The pattern in Myxophaga is also consistent with earlier morphology-based hypotheses (Beutel *et al.*, 1999). A clade Myxophaga excluding Lepiceridae appears well supported by the presence of spiracular gills and ligular papillae of larvae, and also a more or less streamlined body in adults and the loss of the transverse ridge on the metaventrite. Presumptive synapomorphies of Sphaeriusidae and Hydroscaphidae are balloon-shaped spiracular gills, semi-entognathous mouthparts, and rows of lancet-shaped setae on the hind margins of the tergites (Beutel *et al.*, 1999). This seemingly plausible morphology-based scenario is not supported by recent molecular analyses, where Hydroscaphidae are basal, and Lepiceridae and Sphaeriusidae sister taxa (McKenna *et al.*, 2015); D. McKenna, personal communication).

In Polyphaga, the hydrophiloid and staphylinoid terminals are sister taxa and both form a staphyliniform unit. Scirtoidea are monophyletic but are not placed at the base of the suborder, which is suggested by recent analyses of molecular data (Bocak *et al.*, 2014; McKenna *et al.*, 2015). It is apparent that taxon sampling in the megadiverse Polyphaga is too limited. Moreover, it is questionable whether morphological data alone are sufficient to resolve the relationship in such an extremely complex group (see Lawrence *et al.*, 2011).

Morphological versus molecular data

Morphological characters are still an efficient tool to reconstruct phylogenetic relationships, especially when characters of different life stages and organs and body regions are used (e.g. Friedrich & Beutel, 2010; Beutel *et al.*, 2011). Anatomical investigations require specific skills and experience but are certainly less cost-intensive than studies based on transcriptomes and genomes (e.g. Niehuis *et al.*, 2012; Misof *et al.*, 2014). Morphological characters provide an independent dataset for critical evaluations of results based on molecular data, often referred to as a ‘plausiblity check’ by molecular workers (e.g. Misof *et al.*, 2014; Peters *et al.*, 2014), an approach addressed as reciprocal enlightenment by the systematist and dipterist Willi Hennig. It is evident that only morphological characters permit reconstruction of character evolution at the phenotypic level (Beutel *et al.*, 2011; Peters *et al.*, 2014), also including transformation of developmental features. A major point is that only morphological feaures allow for a placement of fossil taxa, at least in invertebrates (e.g. Beutel *et al.*, 2008a, 2013). Furthermore, morphological characters can be gathered from museum specimens unsuitable for obtaining molecular data.

Morphological characters are often subject to homoplasy, resulting from similar patterns of selective pressure, as for instance in the case of independent evolution of mandibular

sucking channels in larvae of the adephagan families Gyrinidae, Haliplidae and Dytiscidae (Beutel, 1993, 1997; Beutel *et al.*, 2013). Another problem can be concerted convergence (Blanke *et al.*, 2012), a phenomenon caused by correlated characters treated as independent features in phylogenetic analyses. Difficulties linked to morphological datasets and their phylogenetic evaluation can be mitigated by the use of a very broad spectrum of characters (e.g. Beutel *et al.*, 2011). However, even the large dataset analysed in the present study (190 characters of adults and immatures) is arguably affected by parallel evolution, especially in the case of Myxophaga and Polyphaga. A clade comprising the two suborders is seemingly supported here by apomorphies of larvae and adults (see also Beutel & Haas, 2000; Beutel *et al.*, 2008a), but not in any of the recently published analyses of molecular data (e.g. Bocak *et al.*, 2014; Misof *et al.*, 2014; McKenna *et al.*, 2015; see also McKenna, 2016).

Early evolutionary history of Coleopterida

The earliest appearance of Coleopterida in the Lower Permian (286 Ma) is suggested by a transcriptomic tree calibrated with fossils (Misof *et al.*, 2014: fig. 2), and a lowermost Mississippian origin (356 Ma, credibility interval 375–336 Ma) was proposed in a recent study where data and topologies of McKenna *et al.* (2015) were recalibrated with fossils (Toussaint *et al.*, 2017). However, at present no fossils documenting the earliest evolution of the lineage are available. The oldest known representatives of Strepsiptera are from Burmese amber (Grimaldi *et al.*, 2005; Engel *et al.*, 2016; Pohl & Beutel, 2016), approximately 100 Ma, more than 200 Myr after the presumptive origin of Coleopterida. The most ancestral representative of the order – †*Protoxenos* Pohl, Beutel & Kinzelbach (Pohl *et al.*, 2005) – is similar to extant strepsipterans like all other described fossils of this group (e.g. Engel *et al.*, 2016; Pohl & Beutel, 2016). Extinct forms sharing features of both orders and possibly belonging in the stem group of Coleopterida are presently unknown.

A crucial event in the evolution of Coleopterida was the development of a posteromotoric flight apparatus. However, this resulted in two very different evolutionary 'strategies' in both lineages. In contrast to the protective elytra of beetles, the forewings of Strepsiptera were transformed into halteres, strikingly similar to those of the anteromotoric Diptera (and the Cretaceous Dipteromantispidae; e.g. Makarkin *et al.*, 2013) and contributing to the excellent flying abilities of the males as gyroscopic balance organs (Pix *et al.*, 1993). The body was light, with a weak degree of sclerotization and a simplified but efficient pterothoracic muscle apparatus (Koeth *et al.*, 2012). A dense vestiture of microtrichia on the cuticle probably improves the aerodynamic properties of the body surface (Pohl & Beutel, 2008). A lack of mechanical protection was apparently irrelevant for the short-lived males with their advanced flight capacity and high manoeuvrability. A crucial question in this context is whether endoparasitim of larvae, the related flightlessness of females, and the short adult life span of males (Kinzelbach, 1971; Pohl & Beutel, 2008) evolved at an early or a later stage of strepsipteran evolution. However, presently no information on this issue is available.

In contrast to Coleopterida, the early evolutionary history of Coleoptera is comparatively well documented in the fossil record (e.g. Ponomarenko, 1969, 1977, 1983, 1995; Kukalová-Peck & Beutel, 2012; Kirejtshuk *et al.*, 2014; Yan *et al.*, 2017a-c; see also Crowson, 1975; Kukalová-Peck, 1991; Rasnitsyn & Quicke, 2002) (Figs 2B, 5), with reliable earliest representatives from the Lower Permian (e.g Ponomarenko, 1969, 1995). All findings of more ancient Carboniferous 'beetles' apparently belong to other insect orders. †*Adiphlebia*, a fossil from the Late Carboniferous (Middle Mississippian) of the U.S.A., was interpreted as the earliest known (Béthoux, 2009). However, this assignment was later refuted (Kukalová-Peck & Beutel, 2012), based on lacking synapomorphies with Coleoptera and a venation showing affinities with that of extant Neuroptera. Another extinct taxon from the Upper Carboniferous, †*Stephanastus* Kirejtshuk & Nel, was placed in Coleopterida as a new monotypic order †Skleroptera (Kirejtshuk & Nel, 2013). The authors point out close phylogenetic affinities with †Umenocoleoidea. The arguments for placing †*Stephanastus* within Coleopterida are unspecific (e.g. small trochanters, hidden coxae), preservation-dependent, and insufficiently documented or not visible at all. The wing venation does not support a placement in Coleopterida, but rather suggests affinities with extinct polyneopteran lineages, such as †Protelytroptera (Haas & Kukalová-Peck, 2001; Rasnitsyn & Quicke, 2002). A close affinity of †Umenocoleoidea with Coleoptera was also suggested by Kirejtshuk & Nel (2013), without presenting specific evidence. However, this group of 'roachoids' belongs to Dictyoptera (Vršanský, 2003), and is apparently closely related to the recently described †Alienopteridae (Bai *et al.*, 2016), both characterized by leathery tegmina and a pronotum transversely subdivided by a supracoxal furrow. Close affinities of the latter group with Mantodea are supported by detailed evidence, including specific features of the head, thorax and genitalia (Bai *et al.*, 2016).

In clear contrast to their sister group, Coleoptera evolved a heavily sclerotized exoskeleton without exposed membranes, with mechanical protection as an obvious benefit, but more or less strongly reduced flying abilities as evolutionary costs. They probably specialized very early on in the penetration of narrow spaces, especially under the bark of conifer trees, which probably also provided shelter and food for the larvae (Beutel, 1997). Associated with this wood-associated lifestyle, a prognathous and wedge-shaped head evolved, and also sclerotized elytra covering the dorsal side of the abdomen and the upper pleural regions and terga of the pterothorax. The most ancestral beetles, †Tshekardocoleidae and †Moravocoleidae (†Protocoleoptera) (Ponomarenko, 1969, 1995; Beutel & Haas, 2000; Kukalová-Peck & Beutel, 2012), were characterized by elytra longer and broader than the abdomen, with window punctures and vestiges of original longitudinal veins, a tuberculate cuticle, a broad prosternal process, a broad prothoracic postcoxal bridge, and possibly 13-segmented antennae (Ponomarenko, 1969; Beutel, 1997). Character transformation in the coleopteran stem group included the formation of elytra adapted

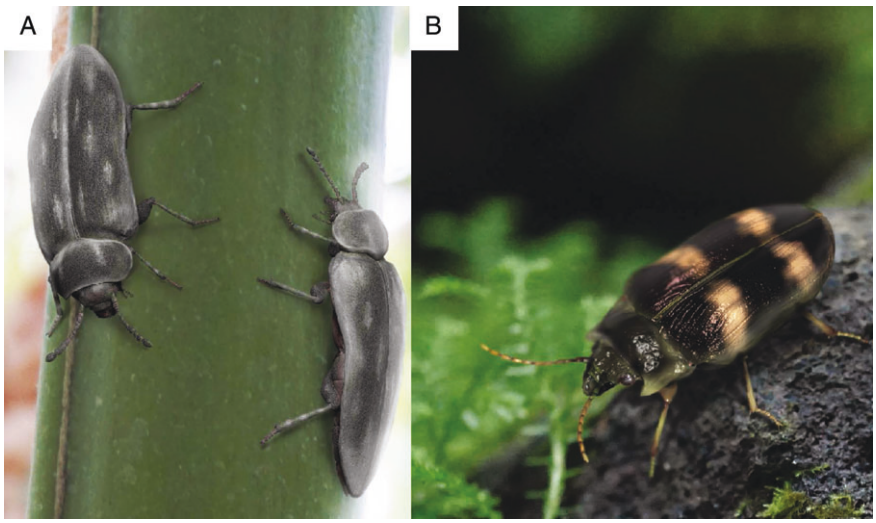

Fig. 5. Late Permian and Late Triassic Coleoptera, habitat reconstruction (from Yan *et al.*, 2017b,c, modifed). (A) †*Ponomarenkia* (Ponomarenkiidae); (B) †*Peltosyne* (Peltosynidae). [Colour figure can be viewed at wileyonlinelibrary.com]

in shape to the abdomen, thus forming a largely closed subelytral space, the narrowing of the prosternal process, the loss of the postcoxal bridge, and possibly the loss of two terminal antennomeres (Beutel, 1997). Plesiomorphies maintained by the extant archostematan families Ommatidae and Cupedidae, but not in any members of the other three suborders, are the tuberculate cuticular surface, the elytral window punctures, the transverse ridge on the mesoventrite, the exposed metatrochantin, and a relatively complex set of thoracic muscles (Beutel & Haas, 2000; Friedrich *et al.*, 2009).

The original reticulate pattern of the elytra was formed by multiplied cross-veins, resulting in a veinal pattern similar to an 'archedictyon', dividing the wing membrane into numerous cells or window punctures. With a reduction in size, the cells were increasingly transformed into vertical supporting structures – columellae. The bottom of these cylindrical (or, in some cases, conical) structures was composed of the wing membrane, whereas its wall involved the walls of surrounding veins (Krüger, 1898; Ponomarenko, 1969, 1983; see also Kirejtshuk *et al.*, 2014).

Whereas a presumably wood-associated ancestral lifestyle was maintained in Archostemata, distinct transitions apparently took place in the Upper Permian and Triassic (Fig. 5A, B). A recently discovered adephagan fossil, probably belonging to the stem group of Gyrinidae, documents an early invasion of aquatic habitats, with specializations such as subdivided compound eyes and a lobe-shaped pedicellus (Yan *et al.*, 2018). An independent invasion of the aquatic environment was probably accomplished in the same period by †Triaplidae (Ponomarenko, 1977, 2016), in very distinct contrast to Gyrinidae characterized by very large metacoxal plates. Later aquatic or semiaquatic groups are the extinct †Catiniidae and †Schizophoridae (Ponomarenko, 1969, 1983), and possibly also †Ademosynidae, which are possibly close to Polyphaga (Yan *et al.*, 2017a). The recently described family †Peltosynidae (Fig. 5B), assigned to the stem group of polyphagans, was certainly terrestrial and probably specialized on wood, as suggested by the large and robust mandibles with molae (Yan *et al.*, 2017b).

Recent investigations of beetle fossils underlined that crucial events in the order took place in the late Permian, prior to the Permian–Triassic mass extinction, which affected beetles (and insects in general) less than other groups of organisms. †*Ponomarenkia* Yan, Lawrence, Beattie, Beutel (Yan *et al.*, 2017c) (Figs. 2B, 5A), one of the very rare Australian beetle fossils of this period (usually only represented by isolated elytra), apparently represents a transitional stage in the group (Fig. 2B). It probably belongs to the crown group of Coleoptera, but to none of the four suborders (Yan *et al.*, 2017c).

The discovery of well-preserved and reliably placed fossils of the stem group of Coleopterida would be as desirable as it appears unlikely. Nevertheless, intensive surveys of Carboniferous, Permian, and also Mesozoic fossils should have high priority. The investigation of tremendously rich but incompletely explored fossil materials (e.g. in Russian, South African and Chinese collections) with modern approaches should be intensified. This concerns not only impression fossils of earlier periods, but also Burmese or even Baltic amber, where evolutionary 'leftovers' like †*Protoxenos* may be preserved. New findings may facilitate the understanding of the apparent conflict between results based either on morphological or molecular evidence. Even though the placement of Strepsiptera in Coleopterida appears to be clarified with different sources of evidence, the early evolution of Coleopterida and Coleoptera is obviously still shrouded in mystery.

Conclusions

The position of Strepsiptera was a matter of long controversy in systematic entomology (Kinzelbach, 1971; Whiting *et al.*, 1997; Wheeler *et al.*, 2001; Wiegmann *et al.*, 2009; Beutel *et al.*, 2011; see also Pohl & Beutel, 2013 and Kjer *et al.*,

2016). It appears now convincingly solved with analyses of large molecular datasets (Niehuis *et al.*, 2012; Boussau *et al.*, 2014; Misof *et al.*, 2014; Peters *et al.*, 2014) and morphological characters (Friedrich & Beutel, 2010; Beutel *et al.*, 2011). A sister-group relationship with a monophyletic Coleoptera is strongly supported by the data presented here. In contrast, the subordinal relationships in Coleoptera remain a challenge, with morphological and molecular data suggesting distinctly different branching patterns. It is conceivable that a denser taxonomic sampling in analyses of transcriptomic data may help to solve this problem. What is suggested by recent analyses of single genes (McKenna *et al.*, 2015) or transcriptomes (e.g. Misof *et al.*, 2014) is clearly in conflict with morphological evidence. The potential of morphological characters appears largely exploited, with well-documented data for different life stages and body regions. In contrast, the exploration of rich fossil sources, especially in collections and fossil sites in Russia and China, still has probably great potential, as shown by recent discoveries of two new families from the late Permian (Yan *et al.*, 2017c) and late Triassic (Yan *et al.*, 2017b). The knowledge of hitherto unknown fossils belonging to the stem group of Coleopterida would probably lead to a better understanding of the evolution of Holometabola. Extinct groups like †Catiniidae or †Schizophoridae, but also recently described new coleopteran taxa (e.g. Yan *et al.*, 2017a,b,c), would probably help to reconstruct early splitting events in Coleoptera, which took place before the Permian–Triassic mass extinction.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1. Complete data matrix: Coleopterida_190charFin.nex.

File S2. Reduced data matrix 1: Coleopterida_156charFin.nex.

Figure S1. Bayesian tree. 190 characters. Posterior probabilities above branches.

Figure S2. Bayesian tree. 156 characters. With 34 characters excluded, all of them presumptive autapomorphies of Strepsiptera (chars. 1, 13, 16, 29, 33, 35, 37, 40, 42, 50, 53, 56, 59, 63, 67–69, 75, 77, 83, 84, 107, 145, 146, 152, 165, 175, 176, 184–188, 190). Posterior probabilities above branches.

Figure S3. Bayesian tree. 190 characters. Monophyletic Coleoptera enforced.

Table S1. Homology of thoracic muscles.

Acknowledgements

We are grateful for helpful comments on this manuscript and technical support from Dr B. Wipfler (Institut für Zoologie und Evolutionsforschung, FSU Jena). The project could be carried out in the framework of the Big4 project. S.-P. Liu was supported from funds of Big4 (Marie-Skłodowska-Curie Innovative Training Networks, EU) and E. Yan by the Alexander-von-Humboldt Foundation. All this is gratefully acknowledged.

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Accepted 7 May 2018

4. Discussion

4.1. The flight mechanism groundplan of Holometabola and the advanced flight apparatuses in "BIG4" orders

It is well known that Holometabola are a group of insects (and organisms) of unparalleled diversity (e.g. Beutel et al. 2017). It was pointed out in different studies that the successful co-evolution with angiosperm plants played a major role (Grimaldi & Engel 2005; Beutel et al. 2017). However, it is also quite obvious that modifications of the flight apparatus played a major role. The small holometabolous orders Raphidioptera, Megaploptera and Mecoptera have largely retained the plesiomorphic pattern of similarly sized pterothoracic segments and wings, without coupling mechanisms connecting the fore- and hindwings (functional quadrupterism).

That functional quadrupterism is also found in distantly related outgroups (e.g. Plecoptera, Zoraptera) suggests that this may be a pleiomorphic pterothoracic configuration in Neoptera. The insects with this flight mode have similar structures of the pterothoracic segments and some very indistinct tergal sulci (Brodsky 1994; Friedrich & Beutel 2010a). These tergal sulci (e.g. scutoscutellar sulcus) enhance the flexible deformation of the tergites to initiate the wing pairs stroking (Brodsky 1994). Meanwhile, their slightly asynchronic wing movements reluctantly support their flight locomotion in a short distance (Brodsky 1994).

In contrast to the flight mechanism in the groundplan of Holometabola, species of the orders of the BIG4 are characterized by functional (Hymenoptera, "higher" Lepidoptera, Coleoptera) or anatomical dipterism (Diptera). Species of Hymenoptera and Diptera almost generally fly exceptionally well, and it is likely that this has contributed to the diversification, as it is almost certainly the case in "higher Lepidoptera" (Study I).

Synchronous depression and asynchronous elevation of wings cause considerable difficulties in insects with functional quadrupterism (Brodsky 1994). In Hymenoptera and "higher" Lepidoptera, the transfer of the independent flight mechanisms of both wing pairs to the mesothorax greatly integrates movements within the flight apparatus (Brodsky 1994). The minute hook shaped hamuli as a hymenopteran autapomorphy link the relatively small hindwings to the forewings in this group (Basibuyuk & Quicke 1997; Grimaldi & Engel 2005). The mesothorax constitutes by far the largest of the three thoracic segments, and the metathorax is reduced to very narrow sclerites with several sclerites absents including basalare and subalare (e.g. honeybee: Snodgrass 1925). A hook-shaped retinaculum arising from the anterior region of the underside of the forewing and a stout bristle (frenulum) form a wing coupling mechanism as a groundplan

apomorphy of the "higher" Lepidoptera subgroup Heteroneura (Kristensen 2003). Compared with the mesothorax, the metathorax of Heteroneura is characterized by the loss of the prescutal arm and a markedly shorted anteromedian part of the metascutum (Study I; Kristensen 2003).

In Diptera, the enlarged mesothorax enhances the anteromotoric flight apparatus increasing speed and power during strokes (Rohdendorf 1974). Correlated with a distinctly reduced metathorax, the hindwings of Diptera are modified as a pair of club-shaped halters. These structures also play an important role as gyroscopic organs during flight (Deora et al. 2017). With an array of campaniform sensilla, the halteres are complex mechanosensory structures that provide sensory feedback to control a stable flight (Agrawal et al. 2017). The mechanosensory signals collected from the halteres are sent to wing steering and head movement motoneurons, allowing direct control the body position and gaze (Yarger & Fox 2016).

An increased flight capacity and maneuverability apparently played a very minor role in the case of the extremely diverse Coleoptera, with very good flight ability only realized in few groups such as for instance tiger beetles (Cicindelinae) or jewel beetles (Buprestidae). A key feature in this order is strong mechanical protection, with sclerotized forewings (elytra) covering the abdomen, reduced degrees of freedom in the thoracic skeleton, and a simplified but fully functional muscular apparatus (Beutel & Haas 2000; Friedrich et al. 2009). Decreasing complexity is the general trend of the thoracic musculature in Coleoptera, accompanied with an increasing efficiency and economy of the locomotor apparatus (Beutel & Haas 2000; Friedrich & Beutel 2010b; Friedrich et al. 2009). A culminating point is reached in the tribe Orectochilini of Gyrinidae, in which only about 20 pterothoracic muscles are preserved in the examined species (Study II). Interestingly, the endoparasitic Strepsiptera, the very small and highly specialized sistergroup of Coleoptera (e.g. Beutel et al. 2011; Peters et al. 2014), have independently evolved anatomical dipterism, however, in contrast to Diptera with functional hindwings (Study V).

4.2. The morphological comparison between the flightless and alate insects

The initial morphological pattern preceding flightlessness in insects requires careful consideration (Wipfler et al. 2015). Some flight-related structural changes might be due to other evolutionary changes or to phylogenetic restraints. The evolution of thoracic structures of a flightless type must be based on a comparison with closely related alate types capable of flight. In Study I, the flightless female of the Japanese wintermoth *N. lefuarius* is compared with alate males with retained flight ability. Compared with the

male *N. lefuarius*, the loss of the tergopleural muscles IItpm4, IIItpm2–5 in both males and females of another geometrid moth *Operophtera brumata* (Linnaues 1758) (Kozlov 1986c), and the loss of the pleuro-coxal muscles II/IIIpcm4, tergopleural muscles IItpm4, IIItpm2–5 in both male and female species of Psychinae (Dierl 1964) are obvious examples. In Study III, it is shown that Hippoboscidae have evolved various modifications of wings including functional and reduced ones (Huston 1984). The thoracic structure of the flightless swift lousefly *C. pallida* is compared with other species of Hippoboscidae with retained flight ability. In Study IV, the modified thoraxes of workers are compared with that of alate queens and other aculeate hymenopteran taxa (Lubbock 1881; Saini 1982). In contrast to the honeybee *Apis mellifica* Linnaeus, 1758, the tergo-pleural muscles IItpm2 and 5 and the sterno-pleural muscles II/IIIspm1 are absent in the queen of *Camponotus camelinus* (Smith 1857) (Saini 1982), and the dorsoventral muscles II/IIIdvm6, the tergopleural muscles IItpm2, 5 and II/IIItpm7 and the sterno-pleural muscles II/IIIspm1 are missing in the queen of *Lasius flavus* (Fabricius, 1782) (Lubbock 1881). These are also examples that flight related structures change for different reasons.

4.3. The trade-off between the flightlessness and the other advantages of insects

As pointed out in the introduction, different factors can lead to secondarily loss of the flight capacity (Table 1). Apparently loss of wings comes at high evolutionary costs, however it can be generally said that loss of the flight capacity is an evolutionary trade off. In secondarily flightless pterygote insects, other parts of the body might be changed to balance the loss of the flight ability (Roff 1986; 1990; Wagner and Liebherr 1992). Berwaerts et al. (2002) have pointed out the flight ability of female butterfly is not as good as the male based on proportionally more mass to the female abdomen. The female *N. lefuarius* (Study I) has greatly enlarged ovaries suggesting enhancement of reproduction. The reduction of the gut indicates that the adults consume no food (Snäll et al. 2007; Wahlberg et al. 2010). In Study III, *C. pallida* displays a compact unit formed by head and thorax, a flattened body, leathery cuticle, posterior shift of dorso-ventral muscles, very movable coxal articulations, short and broad tarsi and specialized claws. These adaptations are linked to ectoparatism (Mossonat 1909; Bequaert 1953; Sclein 1970; Maa & Peterson 1987). Wingless workers of Formicidae treated in Study IV have an enlarged prothorax with the strongly developed skeletomuscular elements, especially muscles associated with the neck, highly fused pterothoracic sclerites and muscles, elongated procoxae with a modified coxo-trochanteral articulation to allow more flexible movements, highly differentiated legs with specialized cleaning devices and attachment structures, and very powerful, specifically arranged muscles of petiole. These structural modifications can assist worker ants to perform different tasks such as lifting or

dragging heavy objects, efficient locomotion on various substrates, keeping sensilla on body surfaces in perfect condition and defending themselves with a highly movable gaster.

Table 1: Modifications/loss of the flight apparatus in the holometabolous orders

	Normal apparatus	**Properties**	**Wing Loss**	**Functional context of winglessness**
Hymenoptera	1) Functional dipterism; 2) Both wing pairs coupled, hind wings reduced in size	High maneuverability	Several families e.g. Mutilidae (females), Formicidae (workers)	1) Sexual dimorphism 2) Division of labor between different castes
Raphidioptera **Megaloptera** **Neuroptera**	Two similarly sized uncoupled pairs of wings	Moderate flight capacity	-	-
Strepsiptera	1) Anatomical dipterism; 2) Forewings transformed into halteres	1) Excellent flying ability; 2) High maneuverability	-	-
Coleoptera	1) Functional dipterism; 2) Forewings transformed into elytra	Mechanical protection	Frequently e.g. *Omus* (Cicindelinae), *Carabus* (partim), Carbidae, Leiodidae, *Meloe* (Meloidae), some Cerambycidae (e.g. *Dorcadion*), etc.	1) Very strong sclerotization; 2) Cave dwelling; 3) parasitisms with increased egg production (*Meloe*) etc.
Trichoptera	Two similarly sized uncoupled pairs of wings	Moderate flight capacity	*Dolophilodes* (Philopotamidae): Females emerging in March are often brachypterous.	Unknown
Lepidoptera	1) basal branches: two similarly sized uncoupled pairs of wings; 2) Heteroneura: functional dipterism	1) basal branches: moderate flight capacity; 2) Heteroneura: excellent flying ability	Several taxa e.g. females of some genera (Geometridae), or all females (Psychinae)	Enhance reproduction
Siphonaptera	Wingless	-	Entire order	Ectoprasitism
Mecoptera	Two similarly sized uncoupled pairs of wings	Moderate flight capacity	Two families e.g. Boreidae, Apteropanorpidae	Adapting to cold temperatures
Diptera	1) Anatomical dipterism; 2) Hind wings transformed into halteres	1) Excellent flying abilities; 2) High maneuverability	Several taxa e.g. Nymphomyiidae, Chironomidae (partim), *Chionea* (Limoniidae); Hippoboscoidea (partim), etc.	1) Development in cold streams, wing shed after eclosion; 2) Surface gliding habits; 3) Preference for cold temperatures

4.4. Flightlessness and the related modifications of flight apparatus

The most obvious change in the muscle system is the modification of the dorsal longitudinal muscle IIdlm1. Compared with its usual condition with several strongly developed parallel bundles in typical alate insects, it has a cotton-like texture in the female of the wintermoth *N. lefuarius* (Study I), it is shorter and narrower in the swift lousefly *C. pallida* (Study III) and completely absent in the worker of *M. nigrocincta* (Study IV). As this muscle normally functions as wing depressor, these changes at least strongly affect the initial contraction of the segment (Kozlov 1986c; Brodsky 1994; Kristensen 2003; Pfau 2008; Deora et al. 2017). The corresponding mesothoracic tergite, pro- and mesothoracic phragmata are deformed. The female *N. lefuarius* (Study I) has a backward shifted recurrent scutoscutellar sulcus compared to the male. This sulcus usually facilitates the deformation of the mesotergite during flight (Brodsky 1994). Both pro- and mesophragma extend anteroventrally in the female, which impedes the normal contraction of the muscle IIdlm1.

The absence of the vertically directed muscles as indirect wing levators (Kozlov 1986c; Brodsky 1994; Pfau 2008; Deora et al. 2017) is apparently correlated with the lost flight ability. In females of the wintermoth *Nyssiodes* (Study I), this concerns the dorsoventral muscles II/IIIdvm1, 3–6, the sterno-pleural muscles II/IIIspm1 and the pleuro-coxal muscles II/IIIpcm2 and 5. In the swift lousefly (Study III), it concerns the dorsoventral muscles II/IIIdvm1, 4 and 5, the sterno-pleural muscle IIspm1 (Friedrich & Beutel 2008). In ant workers (Study IV), the dorsoventral muscles II/IIIdvm1 and 6 and the pleuro-coxal muscles II/IIIpcm2 are reduced. Some of these muscles connecting the wing base sclerites also have other functions in the context flight movements (Brodsky 1994). In *Nyssiodes*, the sterno-pleural muscles II/IIIspm1 and the pleuro-coxal muscles II/IIIpcm2 connected with the basalares are involved in adduction and pronation as secondary functions. S terno-pleural muscle IIspm1 in the swift lousefly (Study III) and dorsoventral muscles II/IIIdvm6 and pleuro-coxal muscles II/IIIpcm2 in ant workers can play a role in different functional contexts.

Secondary flightlessness can also result in deformations of wing base structures, affecting for instance the click mechanism (Brodsky 1994; Pfau 2008; Deora et al. 2017). In Study I, it is shown that the pterothoracic wing base sclerites are deformed in female wintermoths *N. lefuarius*, which consequently loose the function of transmitting force from the thoracic main body to the wing base. A similar condition has evolved in the swift lousefly *C. pallida* treated in Study III, were the mesothoracic wing base sclerites are distinctly reduced. Another structure strongly modified in flightless forms is the pleural

wing process. It has only one head in the female of *N. lefuarius* compared with the two heads in males (Study I), and is reduced to a tiny process in *C. pallida* (Study IV) compared with an elongate rod-shaped structure in other groups of Diptera. Other sclerites such as the basalare and subalare are also more or less deformed in flightless species examined in Study I and III. In Study IV it is shown that the wing base structures of workers of the ant species *M. nigrocincta* are completely absent.

Some flight related structures are retained in secondarily flightless insects as evolutionary relics, apparently as obsolete elements of the thoracic apparatus. Meanwhile, other retained flight related structures have important and specific functions such as providing resiliency for the skeleton or supporting ground locomotion (Kozlov 1986c; Brodsky 1994). In the flightless females of the winter moth *Nyssiodes* (Study I), the laterally bent ventral longitudinal muscles IIvlm3 and IIIvlm2 provide resiliency to support the enlarged ovaries. The dorsoventral muscles II/IIIdvm7 have dual functions involved as flight elevator and walking. The tergo-pleural muscles II/IIItpm4 both control the wing stroke and stabilize the marginal region of the thorax. In ant workers of *M. nigrocincta* (Study IV), the flight elevators II/IIIdvm4, 5 and 7 are even increased in size. This is very likely due to their strong involvement in leg movements and accordingly efficient locomotion on the ground.

An important characteristic of the thoracic locomotor apparatus is that some skeletomuscular elements can take over different functions, for instance support flight movements when other typical elements of the flight apparatus are absent. In Species of Orectochilini treated in Study II have reduced multiple elements of the pterothoracic locomotor system, but nevertheless swim extremely efficiently and have also retained their flight capacity. They use the tergo-pleural muscle IIItpm3 and metapleural muscles IIIppm1 to replace the function of the dorsal longitudinal muscle IIIdlm1, which is normally an essential indirect flight muscle in neopteran insects. Correspondingly, their connecting metabasalare participates in the hindwing depression. Control of the metasubalare is completely undertaken by the tergo-pleural muscle IIItpm10. In other Coleoptera this function depends more on the strongly dorsoventral muscle IIIdvm6.

5. Summary

The acquisition of the wings around in the late Palaeozoic and early Mesozoic is an extreme advantage of Pterygota (Wagner & Liebherr 1992; Beutel et al. 2017). The groundplan of the flight apparatus of adult holometabolan insects is close to that of adult neopteran insects, which have approximately equally sized pterothoracic segments without wing coupling mechanism (Brodsky 1994; Peters et al. 2014). The BIG4 orders referring to the four extremely species rich orders of Holometabola, including Hymenoptera, Coleoptera, Lepidoptera and Diptera, which respectively evolved a more advanced flight apparatus involved in functional or anatomical dipterism. The generally excellent flight capacity assisted the species of Hymenoptera, Diptera and "higher" Lepidoptera to diversify in a very large extent. Meanwhile, the sclerotized forewings of Coleoptera provide strong mechanical protection and represent a confirmed reason of the extreme diversity of this order.

However, in nearly all winged insect orders there are flightless species which lost the immense advantage of flight secondarily (Wagner & Liebherr 1992). Insects living in relatively stable habitats, woodlands and deserts, aquatic environments, cold areas and on animal surfaces are usually flightless (Roff 1986; Wagner & Liebherr 1992). Flightlessness also more frequently happens in females with a sexual dimorphism compared with conspecific males (Roff 1986; Wagner & Liebherr 1992). Previously Wipfler et al. (2015) and Friedrich & Beutel (2010) have initially summarized the general morphological transformations of several flightless members from both Polyneoptera and Holometabola.

The aims of the present study are as followings:

(1) Details of the modified thoracic skeletomuscular structures of flightless members from BIG4 orders are examined and documented with both traditional and advanced morphological techniques, namely histology, hand drawing, microphotography, scanning electron microscopy (SEM) and computer based 3D-reconstruction.

(2) The thoracic skeletomuscular structures of flightless members form the BIG4 orders and those of the phylogenetically related alate individuals are compared anatomically. The functional configurations and interactions of the insect flight apparatus can be more clearly understood.

(3) The well-documented morphological features are crucial to reveal the evolutionary changes based on the phylogenetic patterns. A combination of morphological and

molecular datasets is applied to demonstrate the more detailed evolutionary histories, with morphological transformations and functional implications.

A series of highly qualified anatomically morphological results are well documented with modern techniques for future research. Compared to the traditional methods, the new morphological techniques provide results showing a more detailed impression of habitus and very clearly depicted minute body surface structure (Friedrich et al. 2013; Wipfler et al. 2016). Furthermore, external and internal structures such as sclerites, muscles, nerves and glands are clearly visualized.

The reduced or absent flight related skeletomuscular structures are generally significant thoracic features of flightless insects. In spite of abandon of such a prominent advantage, some modified structures from the other body parts become a trade-off for the adaptation to respectively specialized environments. The enlarged ovaries in the female *N. lefuarius* indicate a higher investment in reproduction. The highly movable coxal articulations and specialized claws of *C. pallida* are linked to ectoparatism. The enlarged prothorax with elongated procoxae, the differentiated legs with cleaning and attachment devices, and the highly movable gaster assist the worker ants to perform different tasks on the ground.

The phylogenetic analyses based on a combination of morphological and molecular datasets clearly demonstrate the evolutionary histories including the enhancement of flight capacity in Lepidoptera, the reduction of thoracic skeletomuscular structures and development of swimming apparatuses in Gyrinidae, the thoracic morphological transformation for ectoparasitism in different families of Hippoboscoidea, and the morphological support to the Coleopterida and its subgroups.

6. Zusammenfassung

Die Evolution der Flügel während des späten Palaeozoikums und frühen Mesozoikums ist ein extremer Vorteil der Pterygota (Wagner & Liebherr 1992; Beutel et al. 2017). Der Grundbauplan des Flugapparats ausgewachsener holometaboler Insekten ist dem adulter Neoptera ähnlich und weist ungefähr gleich große pterotorakale Segmente ohne Kopplungsmechanismen der Flügel auf (Brodsky 1994; Peters et al. 2014). Die „BIG4 orders" umfassen die vier artenreichen Ordnungen der Holometabola und zwar Hymenoptera, Coleoptera, Lepidoptera und Diptera, die jeweils einen fortgeschritteneren Flugapparat, involviert in funktioneller und anatomischer Zweiflügligkeit, evolviert haben. Im Allgemeinen trägt die exzellente Flugkapazität der Hymenoptera, Diptera und „höheren" Lepidoptera signifikant zur Artdiversifizierung dieser Ordnungen bei. Im Gegensatz dazu sind die sklerotisierten Vorderflügel der Coleoptera ein nachgewiesener Grund der extremen Diversität dieser Ordnung, da diese einen mechanischen Schutz liefern.

Allerdings gibt es in allen flügeltragenden Insektenordnungen Arten, die die Fähigkeit des Fliegens verloren haben (Wagner & Liebherr 1992). Hier sind normalerweise Insekten, die in relativ stabilen Habitaten, Wäldern und Wüsten, aquatischen Lebensräumen, kalten Gebieten oder auf der Körperoberfläche anderer Tiere leben, flugunfähig (Roff 1986; Wagner & Liebherr 1992). Flugunfähigkeit tritt sexualdimorphisch häufiger in Weibchen als Männchen der gleichen Art auf (Roff 1986; Wagner & Liebherr 1992). Einen Überblick über die allgemeinen morphologischen Transformationen einiger flugunfähiger Mitglieder der Polyneoptera und Holometabola geben Wipfler et al. (2015) und Friedrich & Beutel (2010).

Die Ziele der vorliegenden Arbeit sind die Folgenden:

(1) Details der abgewandelten skeletomuskulären Strukturen des Thorax flugunfähiger Vertreter der „BIG4 orders" werden unter Nutzung traditioneller und moderner morphologischer Methoden, inklusive Histologie, Zeichnungen, Mikrofotografie, Rasterlektronenmikroskopie (REM) und computer-basierte 3D-Rekonstruktionen, untersucht und dokumentiert.

(2) Die skeletomuskulären Strukturen des Thorax flugunfähiger Vertreter werden mit jenen phylogenetisch nah verwandter geflügelter Individuen anatomisch verglichen. Dies liefert ein besseres Verständnis über die funktionellen Konfigurationen und Interaktionen des Flugapparats der Insekten.

(3) Die gut dokumentierten morphologischen Merkmale sind ausschlaggebend um die evolutionäre Entwicklung, basierend auf phylogenetischen Mustern, nachvollziehen zu können. Die Kombination morphologischer und molekularer Datensätze wurde durchgeführt, um die evolutionäre Geschichte mit morphologischen Transformationen und funktionellen Implikationen zu demonstrieren.

Eine Reihe von qualitativ hochwertigen anatomisch-morphologischen Ergebnissen wurden mit Hilfe moderner bildgebender Verfahren auch für zukünftige Studien dokumentiert. Der Vergleich mit traditionellen Methoden zeigt, dass die neuen morphologischen Ergebnisse einen deutlicheren Einblick in den Habitus und dessen winzige Strukturen geben (Friedrich et al. 2013; Wipfler et al. 2016). Hierbei sind externe und interne Strukturen wie Sklerite, Muskeln, Nerven und Drüsen eindeutig erkennbar und visualisiert.

Die reduzierten oder fehlenden skeletomuskulären Strukturen, die mit der Flugfähigkeit in Zusammenhang stehen, sind ein signifikantes Merkmal flugunfähiger Insekten. Trotz des Verlusts eines solch hervorstechenden Vorteils, gibt es Strukturen anderer Teile des Körpers, die als „trade-off" für die Adaptation an spezielle Lebensräume angepasst sind. Dies sind zum Beispiel: vergrößerte Ovarien der Weibchen von *N. lefuarius*, die auf eine höhere Investition bei der Fortpflanzung hinweisen; stark bewegliche Gelenkungen der Coxa und spezialisierte Klauen von *C. pallida*, die in Verbindung mit Ektoparasitismus stehen; ein vergrößerter Prothorax mit verlängerten Procoxen, differenzierte Beine mit Strukturen für die Reinigung und Anhaftung, und stark bewegliche Hinterleiber, die Arbeiterameisen dabei unterstützen verschiedene Aufgaben auf dem Boden auszuführen.

Die phylogenetischen Analysen, basierend auf der Kombination morphologischer und molekularer Datensätze, veranschaulichen eindeutig die evolutionäre Geschichte inklusive der Steigerung der Flugkapazität der Lepidoptera, die Reduktion der skeletomuskulären Strukturen des Thorax und die Entwicklung des Schwimmapparats der Gyrinidae, die morphologischen Transformationen des Thorax in Bezug zum Ektoparasitismus in verschiedenen Familien der Hippoboscoidea und die morphologische Unterstützung der Coleopterida und deren Taxa.

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8. Appendixes

Study I

Appendix 1: Combined muscular and skeletal data of all studied taxa
Appendix 2: Muscular homology chart
Appendix 3: Characters for mapping analysis
Appendix 4: Character matrix

Study III

Appendix 1: Diptera muscular homology chart. Muscle present is represented by "+" in green, absent by "-" in pink, uncertain by "?" or "/" in yellow.

Study IV

Appendix 1: Combined skeletomuscular characters

Appendix 2: Muscular homology chart of Hymenoptera (Present with "+" or muscular name in green, absent with "-" in pink, uncertain with "?" or "/" in yellow. In Formicidae, the muscles only present in workers are labeled in dark green; muscles only occurring in alate castes labeled in dark blue.)

Study V

Appendix 1: Complete data matrix: Coleopterida_190charFin.nex (See CD-ROM)
Appendix 2: Reduced data matrix 1: Coleopterida_156charFin.nex (See CD-ROM)
Appendix 3: Fig. A1. Bayesian tree 190 characters. Posterior probabilities above branches.
Appendix 4: Fig. A2. Bayesian tree. 156 characters. With 34 characters excluded, all of them presumptive autapomorphies of Strepsiptera (chars. 1, 13, 16, 29, 33, 35, 37, 40, 42, 50, 53, 56, 59, 63, 67-69, 75, 77, 83, 84, 107, 145, 146, 152, 165, 175, 176, 184-188, 190). Posterior probabilities above branches.
Appendix 5: Fig. A3. Bayesian tree. 190 characters. Monophyletic Coleoptera enforced.
Appendix 6: Homology of thoracic muscles

Study I

Appendix 1: Combined muscular and skeletal data of all studied taxa

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Obtectomera

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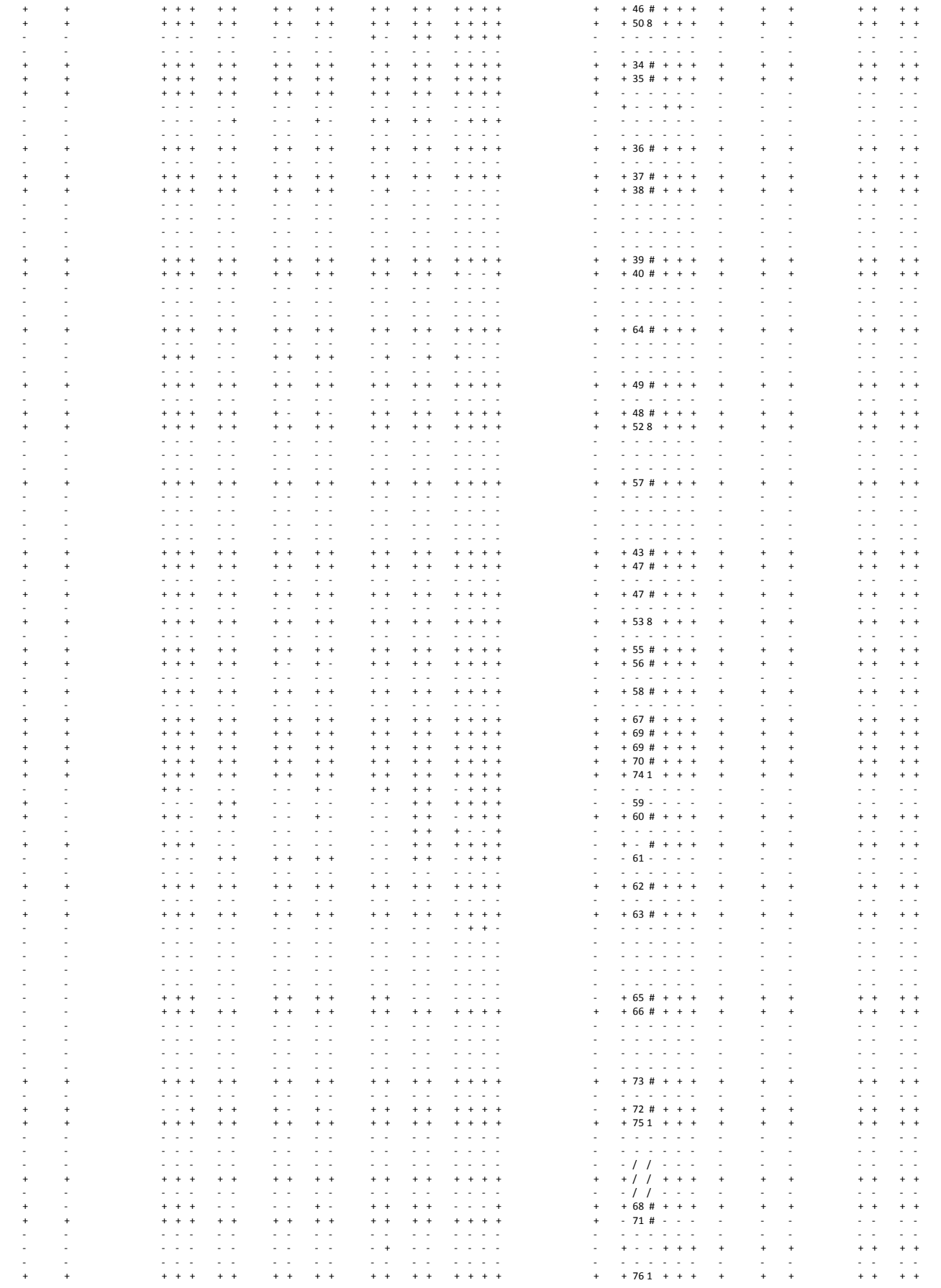

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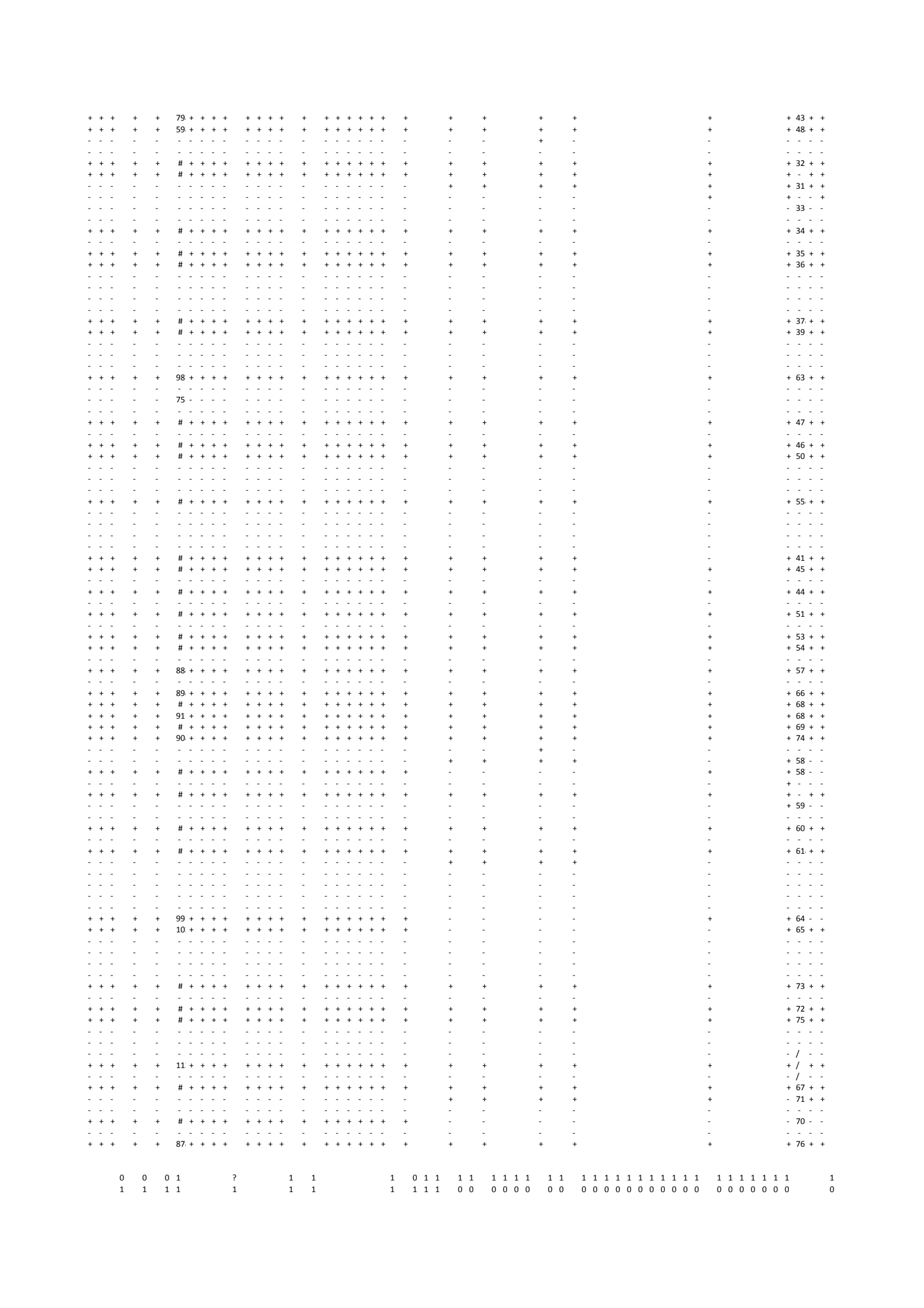

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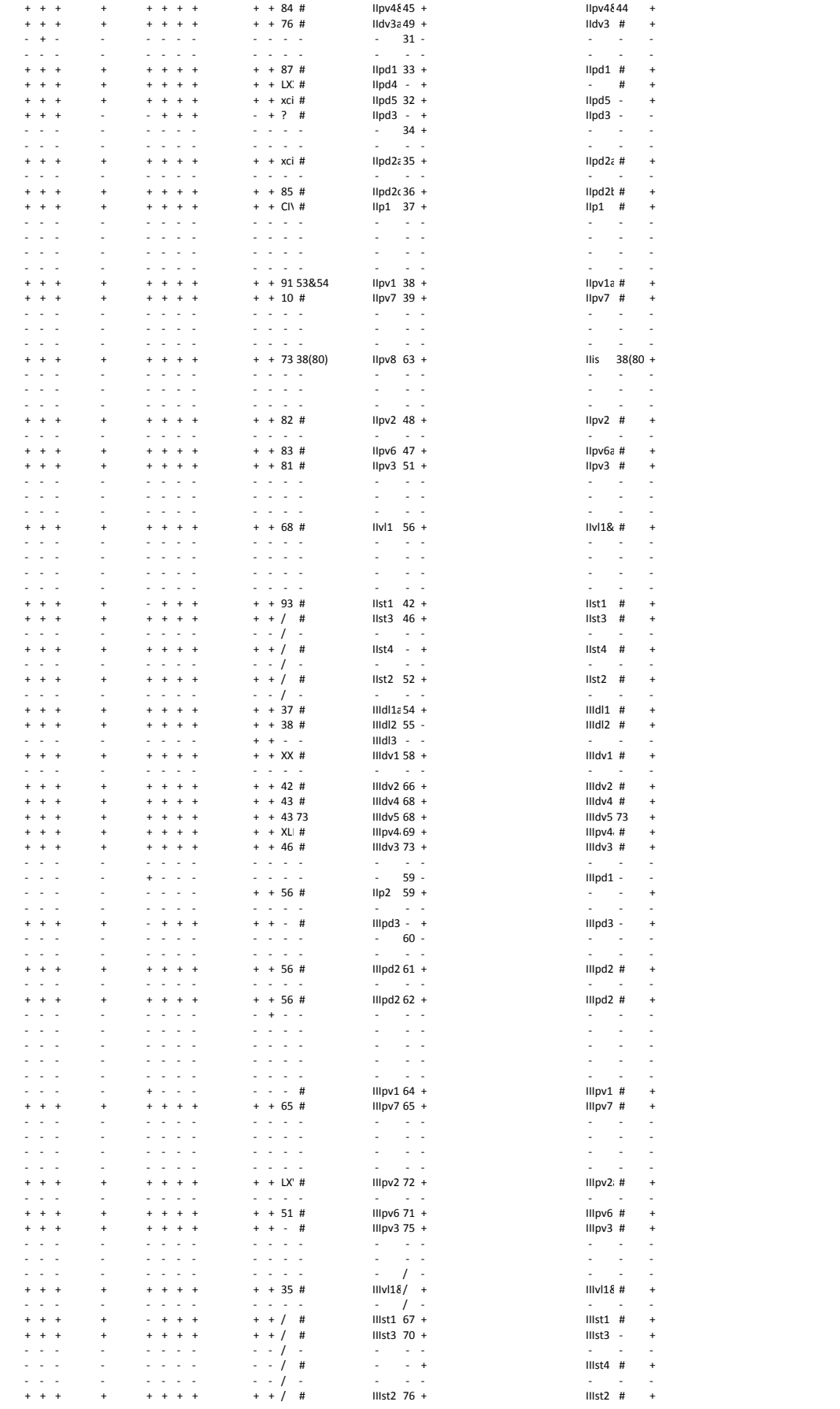

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Study I

Appendix 2: Muscular homology chart

Muscle Name	*N. lefuarus*	Berlese 1909	Bharadwaj *et al.*	Czihak 1956	Dierl 1964 (male	Ehrlich & Davids	Kristensen 2003	Korzeev 2001	Kozlov 2012	Maki 1938 (No. f
Idlm1	+	/	1(1Ph-POc)&2(1L	M.meson.occ.ob	Lbdlm1	30&32	1lph-pocd	CvI.7&CvI.8	/	internal median c
Idlm2	-	/	3(t1-POc)	m pron. occ.	-	29	t1-pocd	CvI.5	/	second anterior c
Idlm3	+	CXI	12(t1-1Ph)&13(t1	Mm. meson.	-	31&35	t1-1lph	/	/	external median
Idlm4	+	/	3(t1-POc)	m pron. occ.	-	28	t1-pocd	CvI.6	/	first anterior dors
Idlm5	+	CXV	12(t1-1Ph)&13(t1	Mm.	Idlm1	33&34	t1-1lph	/	/	external lateral d
Idlm6	+	-	-	Mm.	-	-	-	/	/	-
Idvm1	+	/	9(lcv-POc)	-	Lbism3	19	lcv-poc/tent	CvA.2	/	first anterior inte
Idvm2	+	/	9(lcv-POc)	M. cerv.occ.	-	18	lcv-poc/tent	CvA.3&CvA.4	/	first anterior inte
Idvm3	+	/	9(lcv-POc)	M. cerv.occ.	-	20	lcv-poc/tent	CvA.1&CvI.9	/	second anterior i
Idvm4	+	/	8(t1-lcv)	Mm.	-	21&22	1lph-lcv&t1-tent	CvI.4	/	first anterior inte
Idvm5	-	/	-	M. pron.cerv	-	-	-	-	/	-
Idvm6	-	/	-	-	-	-	-	-	/	-
Idvm7	-	/	-	-	-	-	-	-	/	-
Idvm8	-	/	7(1LPh-lcv)	-	Lbism1	-	t2-lcv&1ipl-tent?	CvP.3	/	second anterior i
Idvm9	+	/	10(fu1-POc)	-	Lbism2	27	fu1-pocd	CvI.3	/	third intersegme
Idvm10	-	-	-	-	-	-	-	/	/	-
Idvm11	-	-	-	-	-	-	-	/	/	-
Idvm12	+	112	18(fu1-1LPh)	-	-	39	fu1-1lph	/	/	posterior tergo-s
Idvm13	-	-	-	-	-	-	-	/	/	-
Idvm14	-	-	-	-	-	-	-	/	/	-
Idvm15	-	-	-	M. pron. cerv.	-	-	-	/	/	-
Idvm16	-	-	-	Mm. pron. cox.	-	-	-	/	/	-
Idvm17	-	-	19 (t1-cx1)	Mm. pron. cox.	-	37	t1-cx1	/	/	tergal remotor (p
Idvm18	-	-	-	-	-	-	-	/	/	-
Idvm19	-	-	-	-	-	-	-	/	/	-
Itpm1	-	/	-	-	-	-	-	-	/	-
Itpm2	-	/	-	-	-	-	eps1-poc/tent&e	CvI.10?	/	-
Itpm3	-	-	-	Mm pron. pl.	-	-	-	/	/	-
Itpm4	+	CXVI&CXVII?	20(t1-pl1(vert))&	Mm pron. pl.	Idvm1	36&40	t1-pl1(vert)&t1-p	/	/	ordinary tergo-pl
Itpm5	-	-	17(plr1-1LPh)	Mm.pron. pl.	lism	-	plr1-1lph	/	/	-
Itpm6	-	-	-	-	-	-	-	/	/	-
1ppm1	-	/	-	-	-	-	-	-	/	-
1ppm2	-	/	-	-	-	-	-	-	/	-
Ispm1	-	-	-	-	-	-	-	/	/	-
Ispm2	-	-	-	-	-	-	-	/	/	-
Ispm3	-	-	-	-	-	-	-	/	/	ventral transvers
Ispm4	-	-	-	-	-	-	-	/	/	-
Ispm5	-	-	-	-	-	-	-	/	/	-
Ispm6	-	-	-	-	-	-	-	/	/	-
Ispm7	-	-	-	-	-	-	-	/	/	-
Ipcm1	+	/	11 (lcv-cx1)	-	Lbism4	23	cx1-lcv&cx1-te&s	CvI.1	/	anterior sternal p
Ipcm2	-	/	-	-	-	-	-	-	/	-
Ipcm3	-	-	-	Mm. pleur.	-	-	-	/	/	-
Ipcm4	+	-	22(pl(eps)1-cx1(r	Mm. pleur. cox.	-	38	pl(eps)1-cx1	/	/	pleural abductor
Ipcm5	+	CXXII	22(pl(eps)1-cx1(r	Mm. pleur. cox.	Ipm1	-	pl(eps)1-cx1	/	/	-
Ipcm6	+	CXXIII	23(pl(epm)1-cx1(	-	-	-	pl('epm')1-cx1	/	/	pleural remotor (
Ipcm7	-	-	-	-	-	-	-	/	/	-
Ipcm8	-	-	-	-	-	-	plr1-tr1dep	/	/	throchanteral mu
Ivlm1	+	/	5(fu1-lcv)&6(fu1-	M. f. cerv.	Lbvlm2&Ivlm3	25&26	fu1-lcv&fu1-prcx	CvP.1&CvP.2	/	longitudinal cervi
Ivlm2	-	/	-	-	-	-	-	-	/	-
Ivlm3	+	/	4(fu1-POc)	Mm. f. tent.	Lbvlm1	24	fu1-pocv/tent&c	CvI.2	/	longitudinal vent
Ivlm4	+	108	16(1sps-fu1)	-	Ivlm2	46	1sps-fu1	/	/	ventral transvers
Ivlm5	-	-	-	-	-	-	-	/	/	-
Ivlm6	-	-	-	-	-	-	-	/	-	-
Ivlm7	+	105&106	14(fu1-fu2)	M. furco-furcalis	Ivlm1	47	fu1-fu2	/	Is13	longitudinal vent
Ivlm8	-	-	-	-	-	-	-	/	-	-
Ivlm9	+	104	15(1sps-fu2)	-	IIvlm3	-	1sps-fu2	/	IIs12	spinofurcal ventr
Iscm1	+	127	24(s1-cx1(adduct	M. st. Cox.	Ibm1&Ibm2?	48	s1-cx1	/	/	ordinary sternal p
Iscm2	+	-	26(fu1-cx1(remo	M. furcopleuro-	-	B	fu1-cx1	/	/	ordinary sternal r
Iscm3	-	-	-	-	-	-	-	/	/	-
Iscm4	+	-	-	M. furcopleuro-	-	41	-	/	/	-
Iscm5	+	-	27(1sps-cx1)	M. mesost.	Ibm3	A	1sps-cx1	/	/	posterior spinal r
Iscm6	-	-	28(plr1-tr1dep)&	M. abd. fem.f.	Ibm4	44	fu1-tr1dep	/	/	trochanteral mus
Iscm7	-	-	-	-	-	-	-	/	/	-
IIdlm1	+	69+70	33(1Ph/sc2-2Ph)	Mm. dors.r	IIdlm1	50&51&52&53&	sc2-2ph	/	IIt14	median dorsal m
IIdlm2	+	71	35(sc2-2LPh)	Mm.dors.obl	IIdlm2	61	sc2-2lph	/	IIt12	lateral oblique do
IIdlm3	+	-	34(Vr2-2PN)	M. scutello-	-	49	vr2-2pn	/	IIt13	median external
IIdvm1	+	LXXVIII	40(t2-peps2)	Mm. scuto-	IIdvm1+2	56&57&58&62	t2-pls2	/	IIt-s12/13	anterior tergo-ste
IIdvm2	-	-	-	-	-	-	-	/	-	-
IIdvm3	+	74	41(t2-cx2)	M.sc.	IIdvm3	63	t2-cx2	/	IIt-ti2	tergal promotor (
IIdvm4	+	75	43(a)(t2-me2)	Mm.sc.merales	IIdvm5	65	t2-me2(ext)	/	IIt-cx7	tergal remotor (n
IIdvm5	+	75	43(b)(t2-me2)	Mm.sc.merales	IIdvm5	60	t2-me2(int)	/	IIt-cx7	tergal remotor (n
IIdvm6	+	84	44(sa2-me2)	M. subal.mer.	IIpm8	79&80	sa2-me2	/	IIp-cx10	coxo-subalar mus
IIdvm7	+	76+77	42(t2-tr2)	M.abd.fem.sc.	IIdvm4	59&64	t2-tr2	/	IIt-tr1	tergal depressor
IIdvm8	-	-	37(fu2-2LPh)	-	-	-	fu2-2lph	/	IIt-s1	posterior tergo-s
IIdvm9	-	-	-	-	-	-	-	/	-	-
IItpm1	+	87	46(sc2-ba2(horiz	Mm.n.epist.?	IIpm3	68	sc2-ba2(ant)	/	IIt-p3	second ordinary t
IItpm2	+	LXXVI	48(sc2-tga2)	Mm.n.epist.?	IIpm4	67	sc2-tpa2	/	IIt-p4	second ordinary t
IItpm3	+	xci	47(sc2-ba2(vert.)	Mm.n.epist.?	IIpm5	-	sc2-ba2(post)	/	IIt-p7	first ordinary terg
IItpm4	+	?	50(plr2-lax2)	-	-	78A	plr2-1ax2	/	IIt-p10	-
IItpm5	-	-	50(plr2-sc2)	M.sc.pleurocost.	-	-	plr2-sc2	/	IIt-p12	third ordinary ter
IItpm6	-	-	-	m.pleurocost.sc.	-	-	-	/	-	-
IItpm7	+	xcii	51(eps2-3ax2)	M. epis.al.	IIpm6	78	eps2-3ax2	/	IIt-p13	first pleuro-axilla
IItpm8	-	-	-	-	-	-	-	/	-	-
IItpm9	+	85	52(epm2(plr2)-3a	M.	IIpm7	82	plr2-3ax2	/	IIt-p14	second pleuro-ax
IItpm10	+	CIV	49(sc2-epm2)	M. epim. subal.	-	81	sa2-epm2	/	IIp6	pleuro-subalar m
IItpm11	-	-	-	-	-	-	-	/	-	-
IItpm12	-	-	-	-	-	-	-	/	-	-
IIppm1	-	-	-	M. intraepist.	-	-	aeps2-peps2(lat)	/	-	pleural muscle (T
IIppm2	-	-	-	-	-	-	-	/	IIp2	-
IIspm1	+	91+91a	53&54(aeps2/ba	-	IIpm1	69	ba/aeps2-peps2(	/	IIp3	sterno-basalar m
IIspm2	+	100	45(fu2-plr2)	M. f pleurocost.	IIzm	84	fu2-plr2	/	IIp-s1	furco-entopleura
IIspm3	-	-	-	-	-	-	fu2/isg-ba3?	/	-	-
IIspm4	-	-	-	-	-	-	-	/	-	-
IIspm5	-	-	-	-	-	-	-	/	-	-

IIspm6	+	73a	38(80)(fu2-ba3/a	M.	-	98	fu2/isg-ba3	/	IIp-s3	first sterno-basal
IIspm7	-	-	-	-	-	-	1ipl-fu2	/	-	-
IIspm8	-	-	-	-	-	75.1	-	/	IIp-s5	-
IIpcm1	-	-	-	-	-	-	-	/	-	-
IIpcm2	+	82	55(ba2-cx2)	-	-	70	ba2-cx2	/	IIp-cx3	coxo-basalar mus
IIpcm3	-	-	-	M. epist.cox.l.	-	-	-	/	-	-
IIpcm4	+	83	56(peps2(aeps2)	M. epist.cox. br.	-	83	eps2-cx2	/	IIp-cx4	pleural abductor
IIpcm5	+	81	60(ba2-tr2dep)	-	IIpm2	71	ba2-tr2	/	IIp-tr2	trochantero-basa
IIpcm6	-	-	-	M.abd.fem.epist	-	-	-	/	-	-
IIvlm1	-	-	-	-	-	-	-	/	-	-
IIvlm2	-	-	-	-	-	-	-	/	-	-
IIvlm3	+	68&104?	36(fu2-fu3)	M. furco-furcalis	IIvlm1&IIvlm2	85	fu2-fu3	/	IIs13	longitudinal vent
IIvlm4	-	-	-	-	-	-	-	/	-	-
IIvlm5	-	-	-	-	-	-	2sps-fu3	/	-	-
IIvlm6	-	-	-	-	-	-	-	/	-	-
IIvlm7	-	-	-	-	-	-	-	/	-	-
IIscm1	+	93	57(s2-cx2)	M. st. Cox.ant.	IIbm1	55	s2-cx2	/	IIs-cx5	ordinary sternal p
IIscm2	+	/	59(fu2-me2(rem	M. st. Cox.pos.	-	75	fu2-me2	/	IIs-cx6	ordinary sternal r
IIscm3	-	/	-	-	-	-	-	/	-	-
IIscm4	+	/	58(fu2-cx2lat/me	-	-	66	fu2-cx2	/	IIs-cx2	ordinary sternal r
IIscm5	-	/	-	-	-	-	-	/	-	-
IIscm6	+	/	61(fu2-tr2dep)	m.abd.fem.f.	IIbm2	77	fu2-tr2dep	/	IIs-tr1	sternal depressor
IIscm7	-	/	-	-	-	-	-	/	-	-
IIIdlm1	+	37	64(2Ph/sc2-3Ph)	Mm. dors.r	IIIdlm1	93	2ph-tI	/	IIIt14	median dorsal m
IIIdlm2	+	38	66(Vr3-3LPh)	Mm.dors.obl	IIIdlm2	92	sc3-3lph	/	IIIt12	lateral oblique dc
IIIdlm3	-	-	65(sc3-3LPh)	-	-	-	vr3-3pn	/	IIIt13	-
IIIdvm1	+	XXXVI	70(t3-peps3)	Mm. scuto-	IIIdvm1	88&94	t3-pls3	/	IIIt-s12/13	anterior tergo-st
IIIdvm2	-	-	-	-	-	-	-	/	-	-
IIIdvm3	+	42	71(t3-cx3)	M.sc.	IIIdvm2	89&95	t3-cx3	/	IIIt-ti2	tergal promotor (
IIIdvm4	+	43	73(t3-me3)	Mm.sc.merales	IIIdvm4	91	t3-me3	/	IIIt-cx7	tergal remotor (r
IIIdvm5	+	43	73(t3-me3)	Mm.sc.merales	IIIdvm4	91	t3-me3	/	IIIt-cx7	tergal remotor (r
IIIdvm6	+	XLIX	74(sa3-me3)	M. subal.mer.	IIIpm6	97	sa3-me3	/	IIIp-cx10	coxo-subalar mus
IIIdvm7	+	46	72(t3-tr3)	M.abd.fem.sc.	IIIdvm3	90&96	t3-tr3	/	IIIt-tr1	tergal depressor
IIIdvm8	-	-	69(fu3-3LPh)	M.	-	-	fu3-3lph	/	IIIt-s1	posterior tergo-s
IIItpm1	+	-	76(sc3-tga3)	Mm.n.epist.?	IIIpm3	-	sc3-ba3(ant)	/	IIIt-p3	first tergo-pleura
IIItpm2	+	56a	39 (fu2-plr3)&76(	Mm.n.epist.?	-	112	sc3-tpa3	/	IIIt-p4	first tergo-pleura
IIItpm3	+	-	-	Mm.n.epist.?	-	-	sc3-ba3(post)	/	IIIt-p7	-
IIItpm4	+	-	77 (plr3-lax3)	-	-	102	plr3-1ax3	/	IIIt-p10	second tergo-ple
IIItpm5	+	-	77 (plr3-sc3)	M.sc.pleurocost.	-	-	plr3-sc3	/	IIIt-p12	third tergo-pleur
IIItpm6	-	-	-	m.pleurocost.sc.	-	-	-	/	-	-
IIItpm7	+	56	78 (eps3-3ax3)	-	IIIpm4	110	eps3-3ax3	/	IIIt-p13	first pleuro-axilla
IIItpm8	-	-	-	-	-	-	-	/	-	-
IIItpm9	+	56	79 (epm2(plr2)-3	M.	IIIpm5	111	plr3-3ax3	/	IIIt-p14	second pleuro-ax
IIItpm10	-	-	-	M. epim. subal.	-	-	sa3-epm3	/	IIIp6	-
IIItpm11	-	-	-	-	-	-	-	/	-	-
IIItpm12	-	-	-	-	-	-	-	/	-	-
IIIppm1	-	-	-	M. intraepist.	-	-	-	/	-	pleural muscle (T
IIIppm2	-	-	-	-	-	-	-	/	-	-
IIIspm1	+	-	81(ba3-peps3)	-	IIIpm1	99	ba/aeps3-peps3(	/	IIIp2	second sterno-ba
IIIspm2	+	65	75(fu3-plr3)	M. f pleurocost.	IIIzm	103	fu3-plr3&fupr3-fi	/	IIIp-s1	furco-entopleura
IIIspm3	-	-	-	-	-	-	-	/	-	-
IIIspm4	-	-	-	-	-	-	-	/	-	-
IIIspm5	-	-	-	-	-	-	-	/	-	-
IIIpcm1	-	-	-	-	-	-	-	/	-	-
IIIpcm2	+	LXVII	82(ba3-cx3)	-	-	100	ba3-cx3	/	IIIp-cx3	coxa-basalar mus
IIIpcm3	-	-	-	M. epist.cox.l.	-	-	-	/	-	-
IIIpcm4	+	51	83(pep3(aeps3)-(	M. epist.cox. br.	-	109	eps3-cx3	/	IIIp-cx1/4	pleural abductor
IIIpcm5	+	-	87(ba3-tr3dep)	-	IIIpm2	101	ba3-tr3	/	IIIp-tr2	trochantero-basa
IIIpcm6	-	-	-	M.abd.fem.epist	-	-	-	/	-	-
IIIpcm7	-	-	-	-	-	-	-	/	-	-
IIIvlm1	-	-	-	-	-	-	-	/	-	/
IIIvlm2	+	35	67&68(fu3-sIIA)	-	IIIvlm1&IIIvlm2	113&114	fupr3/fuse3-sII	/	IIIs20	/
IIIvlm3	-	-	-	-	-	-	-	/	-	/
IIIscm1	+	/	84(s3-cx3)	M. st. Cox.ant.	IIIbm1	86	s3-cx3	/	IIIs-cx5	sternal promotor
IIIscm2	-	/	86(fu3-me3(rem	M. st. Cox.pos.	-	-	fu3-me3	/	IIIs-cx6	sternal remotor (
IIIscm3	-	/	-	-	-	-	-	/	-	-
IIIscm4	-	/	85(fu3-cx3lat/me	-	-	108	fu3-cx3	/	IIIs-cx2	sternal remotor (
IIIscm5	-	/	-	-	-	-	-	/	-	-
IIIscm6	+	/	88 (fu3-tr3dep)	-	IIIbm2	87&107	fu3-tr3dep	/	IIIs-tr1	sternal depressor

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Matsuda 1956	Matsuda 1970 (L	Mitchell & Seabr	Nuesch 1953; Ma	Srivastava 19618	Tindall 1965	Vilhelmsen 2000	Wittig 1955
6	op-t3	I27	Id1&Id3	30&31	I1.01-1.08&I1.51	4	I dlm 10
5	op-t2	I25?&I26?	Id2&Id5	29	I1.55	-	0 dlm 1
7+8	t14&cv(d)-t1	I30	Idl1	38&39	I1.54	-	I dlm 11b
4	op-t1	?	Id7	28	I1.56	-	0 dlm 2
10	t12	I29	Idl2/Idl2a&b	40&41	I1.09	19	I dlm 12
9	t13	-	-	-	I1.10&I1.11	-	I dlm 12?
-	op-cv1	I21	Id4/Id4a	25?	I3.03	-	0 lm 7
2?	op-cv2	I22	Id4b	26	I3.04	1	0 lm 7
2?	op-cv3	I23	Id4c	27?	-	1	0 lm 8
1	t-s(cv)1	-	Id8	34	I3.01	-	0 lm 5
-	t-cv1	-	-	-	-	-	-
-	t-cv2	-	-	-	I3.02	5	0 lm 6
-	-	-	-	-	-	8?	-
-	t-s(cv)9&p-s(cv)7	-	Icd	-	I4.10	8?	-
-	op-s2	I28	Id6	32	I3.05	2	-
-	t-s1	-	-	-	I3.51	20	I ism 22
-	-	-	-	-	-	-	I ism 24
40&41	t-s2	I32	Idv/Iis	44	-	-	-
14?	-	-	-	-	-	-	I dvm 15
14?	-	-	-	-	-	-	I dvm 16
18	-	-	-	-	-	-	I dvm 17?
15	-	-	-	-	-	11?	I dvm 19
16	t-cx7	-	-	45	I7.52	11?	I dvm 20
17	-	-	-	-	-	-	I dvm 21
20	-	-	-	-	-	-	I dvm 18
12?	-	-	-	-	-	-	-
11	op-p1	?	It/It1	-	-	-	0 lm 9
13	t-p (proth.)	I31	-	-	I4.03&I4.04	9	I tpm 25
-	t-p (proth.)	I33a&b	Ipda&b	42&43	I4.05&I4.06&I4.0	10	I tpm 26
-	t-p (proth.)	-	Ipdc/Ipdc&d&e	-	I4.08	-	I tpm 27?
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	?	I zm 34
-	p-s2	-	-	-	-	?	-
-	p-s6	-	-	-	-	?	-
-	-	-	-	-	-	?	-
-	-	-	-	-	-	?	-
-	-	-	-	-	-	?	-
-	-	-	-	-	-	?	-
-	cv-cx3&s-cx(cv)1	I18?	It2	49	-	?	-
-	-	-	-	-	-	7	-
-	-	-	-	-	-	18?	-
19	p-cx4	I38&I39	-	48	I7.03	12	-
-	p-cx5	I34	Ipv6/Ipv6a&b&c	-	I7.04	-	I cpm 28
-	p-cx9	-	Ipva	-	I7.51	14	-
-	-	-	-	-	-	-	-
21?	p-tr1	-	-	-	-	17?	I cpm 29
3	cv-s1/4	I20&I24?	Icv/Icv1&Icv2	35&36	I2.51	6	0 vlm 4
-	-	-	-	-	-	-	-
26&27	s1/2	I19?	Iv	33	I2.01&I2.02	3	0 vlm 3
-	s14	I41	Ivl2	37	II2.01	23	I vlm 14
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
57	s13	I45a&b	Ivl1	68	II2.51	21&22	I vlm 13
-	-	-	-	-	-	-	-
-	s12	II41	Ilvl2	-	II2.02	24	-
25	s-cx5	I37&I43&I44	Ista&st1	50&51	I7.01&I7.02	13	I bm 30
24	s-cx3	I36&I42	Istb	47	I7.53&I7.54	14part	I bm 33
23	-	-	-	-	-	-	I bm 32
-	-	I35	Istd	46	-	15	-
-	s-cx4	I40	Istc	52	I7.55?&I7.56	16	-
-	s-tr1	-	-	55a	I8.11	17	I bm 31
-	-	-	-	-	-	-	-
30	t14	II75	IIdl1a-e	65&66&67	II1.01-1.05	?	II dlm 35
31	t12	II71	IIdl2	70	II1.57	?	II dlm 36&II dlm
-	t13	-	IIdl3	64	II1.54	?	-
43	t-p5/6	II53a&b	IIdv1a&b	69	II3.03	?	II dvm 40
32?	-	-	-	-	-	?	II dvm 41
32?	t-ti(cx)2/3	II54	IIdv2	79	II3.04	?	-
33&34	t-cx6	II56	IIdv4	83	II7.52	?	II dvm 43
35	t-cx7	II57	IIdv5	83	II7.52	?	II dvm 43
37	t-cx8	II49a&b	IIpv4&IIpv5	82	II5.51	?	II cpm 53
48	t-tr1	II55a&b	IIdv3a&b/IIdv3	87b&c	II8.01&II8.02	?	II dvm 42
-	t-s1	II77	-	-	-	?	II ism 44
-	-	-	-	-	-	?	-
44	t-p3	II58	IIpd1	71	-	?	II tpm 46a
42?	t-p4	II65	IIpd4	77	II4.01	?	II tpm 47
45	t-p7	II61	IIpd5	-	II5.01	?	II tpm 46b?
51	t-p10	II60	IIpd3	-	-	?	-
-	t-p12	-	-	-	-	?	-
-	t-p15	II76?	-	-	II4.02	?	II tpm 49
-	t-p13	II59a	IIpd2a&b/IIpd2a	75	II6.01	?	II tpm 48
-	-	-	-	-	-	?	-
-	t-p14	II59b	IIpd2c/IIpd2b	74	-	?	-
-	t-p16	II69	IIp1	76	II5.52	?	II ppm 56
-	-	-	-	-	-	?	-
-	-	-	-	-	-	?	-
-	p1	-	-	-	-	?	II im 65a
-	p2	-	-	-	-	?	II pm 54a&b
-	p3	II46	IIpv1/IIpv1a&b&I	72	-	?	II ppm 55
-	p-s1	II51	IIpv7	73	II4.51&II4.52	?	II zm 61a
-	-	-	-	-	-	?	-
-	-	-	-	-	-	?	-
-	-	-	-	-	-	?	-

-	p-s3	II52	IIpv8/IIis	106	III9.03?	29	-
-	p-s9	-	-	-	-	?	-
-	p-s5	-	-	-	III9.03?	?	-
-	-	-	-	-	-	?	-
50	p-ti(cx)2/3	II47	IIpv2	80	II5.02	?	II cpm 51
-	-	-	-	-	-	?	-
36	p-cx5	II50	IIpv6/IIpv6a&b	81	-	?	II cpm 52
47	p-tr2	II48	IIpv3	87a	II8.03	?	II cpm 50
-	-	-	-	-	-	?	-
59	-	-	-	-	-	?	-
-	-	-	-	-	-	?	-
-	s13	II45a&b	IIvl1/IIvl1&IIvl2	97	III2.01&III2.02	27	II vlm 38
-	-	-	-	-	-	?	-
-	s12	-	-	-	-	28	II vlm 39
-	-	-	-	-	-	?	-
-	-	-	-	-	-	?	-
56	s-cx5	II70	IIst1	78	II7.11&II7.12	?	II bm 57
54	s-cx3	II67	IIst3	85	II7.13	?	II bm 60
-	-	-	-	-	-	?	II bm 59
55	s-cx2	II68	IIst4	84	II4.53	?	II zm 61b
-	-	-	-	-	-	?	-
-	s-tr1	II66	IIst2	87d	II8.11	?	II bm 58
-	-	-	-	-	-	?	-
63	t14	III75	IIIdl1a&b/IIIdl1	99	III1.01	5	III dlm 35
64	t12	III71	IIIdl2	100	III1.57	6	III dlm 36&III
-	t13	-	IIIdl3	-	III1.54	?	-
71	t-p5/6	III53	IIIdv1	98	III3.03	9	III dvm 40
65?	-	-	-	-	-	17	III dvm 41
65?	t-ti(cx)2/3	III54	IIIdv2	109	III3.04	-	-
66	t-cx6	III56	IIIdv4	113	III7.52	18	III dvm 43
67	t-cx7	III57	IIIdv5	113	III7.52	19	III dvm 43
69	t-cx8	III49a&b	IIIpv4&IIIpv5	112	III5.51	22	III cpm 53
74	t-tr1	III55	IIIdv3	116b&c	III8.01&III8.02	20?	III dvm 42
-	t-s1	-	-	-	-	7&8	III ism 44
72	t-p3	-	IIIpd1	-	-	3	III tpm 46a
-	t-p4	-	IIp2	107	III4.01	4	III tpm 47
73	t-p7	-	-	-	III5.01	11	III tpm 46b
76	t-p10	III60	IIIpd3	103	III4.02	-	-
-	t-p12	-	-	-	-	10	-
-	t-p15	III76?	-	-	-	-	III tpm 49
-	t-p13	III59b	IIIpd2b/IIIpd2a	104	III6.01	12a	III tpm 48
-	-	-	-	-	-	-	-
-	t-p14	III59a&c	IIIpd2a&c/IIIpd2b	105	-	12b	-
-	t-p16	-	-	-	III5.52	15	III ppm 56
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	p1	-	-	-	-	14?	III im 65a
-	p2	-	-	-	-	-	III ppm 54a&b
-	p3	III46	IIIpv1	101	-	13	III ppm 55
+	p-s1	III51	IIIpv7	102	III4.51	23	III zm 61
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
78	p-ti(cx)2/3	III47	IIIpv2/IIIpv2a&b	110	III5.02	-	III cpm 51
-	-	-	-	-	-	21?	-
68	p-cx5	III50	IIIpv6	111	-	21?	III cpm 52
75	p-tr2	III48	IIIpv3	116a	III8.03	26	III cpm 50
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
81	-	-	-	/	-	-	-
80	s20	III41&III45	IIIvl1&IIIvl2	/	III3.51	-	III vlm 64
-	-	-	-	/	-	35	-
+	s-cx5	III70	IIIst1	108	-	-	III bm 57
+	s-cx3	III67	IIIst3	114	III7.13	25	III bm 60
-	-	-	-	-	-	30	III bm 59
+	s-cx2	-	IIIst4	-	-	-	-
-	-	-	-	-	-	24	-
-	s-tr1	III66	IIIst2	116d	III8.11	-	III bm 58

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Study I

Appendix 3: Characters for mapping analysis

	No.	Original No.	Source	Character	0	1	2
Prothorax	**1**	366	Heikkilä et al. 2015	Cervical sclerites	not joined ventromedially strong	joined ventromedially above :	join at sternum
	2	367	Heikkilä et al. 2015	Cervical sclerites with strong inward turn	absent (0)	present (1)	
	3	13	Nielsen & Kristensen 1996		Posterior corner of laterocervical	distinctly dorsad to anepisternal	tooth/pleurocoxal
	4	369	Heikkilä et al. 2015	Strong reduction or loss of the ventral arms of tl	absent (0)	present (1)	
	5	12	Nielsen & Kristensen 1	Proprioceptive setae on apical arm of laterocerv	present (0)	absent (1)	
	6	358	Heikkilä et al. 2015	Patagia	absent or present at most as a tu	present as a distinct flap (1)	present as somewhat
	7	359	Heikkilä et al. 2015		Pronotum without lateral extensi	with elongated lateral arms which extend into pata	
	8	360	Heikkilä et al. 2015	Parapatagia distinct lobes	absent (0)	present (1)	
	9	14	Nielsen & Kristensen 1	Anteromedian pronotal sclerite that articulates '	absent (0)	present (1)	
	10	15	Nielsen & Kristensen 1	Pro-precoxal bridge	absent (0)	present (1)	
	11	17	Nielsen & Kristensen 1	Base of free profurcal arm	distinctly separate from pleural h	touching or more or less extensively fused with pleu	
Mesothorax	**12**	361	Heikkilä et al. 2015	Dorsal extremities of prescutal clefts on mesoth	relatively close clefts obliquely di	relatively great length betwee	clefts meet or nearly
	13	379	Heikkilä et al. 2015	Ventral processes of tegula	blunt (0)	sharp (1)	elongated spatulate (2
	14	370	Heikkilä et al. 2015	Anepisternum of mesothorax	well developed (0)	reduced to a tiny sclerite or absent (1)	
	15	371	Heikkilä et al. 2015	Precoxal suture	absent (0)	present (1)	
	16	372	Heikkilä et al. 2015	Parepisterna partly concealing margin of basiste	absent (0)	present (1)	
	17	373	Heikkilä et al. 2015	Parepisternal suture	absent or only partly present (0)	well-developed all the way from the basisternum to	
	18	375	Heikkilä et al. 2015	Secondary sternopleural suture	absent (0)	present (1)	partly as non-sclerotiz
	19	383	Heikkilä et al. 2015	Mesothorax, line of junction on meron and epim	short (0)	long (1)	midway (2)
	20	384	Heikkilä et al. 2015	Suture in shape of Y on mesepimeron	absent (0)	present (1)	
	21	21	Nielsen & Kristensen 1	Anterodorsal marginal area of mesepimeron	simple (0)	anteriorly curling, concealing upper portion of pleur	
	22	2	Sharplin 1964	Median notal wing process (Posterior)	Median notal wing process and fi	Median notal wing process, a	Large median notal w
	23	4	Sharplin 1964	Junction between the radial plate and the bases	No joints present (0/G)	One joint present between th	Both folding points pr
	24	9	Sharplin 1964	Flexible cuticle in the wing base	absent (0/-)	Small mesocuticular pegs, irre	In at least one area of
	25	10	Sharplin 1964		a broad bending cuticle connecti	No ventral bending cuticle in the mesothorax; prese	
	26	377	Heikkilä et al. 2015	Spinasternum bifurcate	absent (0)	present (1)	
	27	378	Heikkilä et al. 2015	Spinasternum sclerotized between the bifurcati	absent (0)	present (1)	
	28	19	Nielsen & Kristensen 1	Anteromedial margin of mesobasisternum	straight or with weakly convex cu	markedly produced anteriorly	markedly produced, a
	29	20	Nielsen & Kristensen 1	Transverse basisternal sutures, branching off fro	absent (0)	present (1)	
	30	380	Heikkilä et al. 2015	Mesophragma	without dorsal processes (0)	with dorsal processes (1)	with flat ridges (2)
	31	381	Heikkilä et al. 2015	Slit on mesophragma reaches well up onto dorsa	no (0)	yes (1)	
	32	18	Nielsen & Kristensen 1	Mesophragma index	low, < 0.45 (0)	high, > 0.55 (1)	
	33	386	Heikkilä et al. 2015	Fusion of mesothoracic lamella	not all the way up the mesofurca	all the way up the mesofurca (1)	
	34	387	Heikkilä et al. 2015	Mesothoracic furca with lateral extensions	no (0)	yes (1)	
	35			IIdlm2	absent (0)	present (1)	
	36			IIdlm3	absent (0)	present (1)	
	37			IIdvm8	absent (0)	present (1)	
	38			IItpm2	absent (0)	present (1)	
	39			IItpm3	absent (0)	present (1)	
	40			IItpm4	absent (0)	present (1)	
	41			IItpm5	absent (0)	present (1)	
	42			IItpm6	absent (0)	present (1)	
	43			IItpm10	absent (0)	present (1)	
	44			IIppm1	absent (0)	present (1)	
	45			IIspm1	absent (0)	present (1)	
	46			IIspm8	absent (0)	present (1)	
	47			IIpcm2	absent (0)	present (1)	
	48			IIpcm4	absent (0)	present (1)	
	49			IIscm1	absent (0)	present (1)	
	50			IIscm2	absent (0)	present (1)	
	51			IIscm4	absent (0)	present (1)	
Metathorax	**52**	5	Sharplin 1964	Metaprescutal arm	a lateral extension of the metapr	Prescutal arm, apparently absent, but probably fuse	
	53	396	Heikkilä et al. 2015	Division of metascutum	not divided mesally (0)	divided mesally (1)	
	54	407	Heikkilä et al. 2015	Metathorax patch of microtrichial spines aculei (	absent (0)	present (1)	
	55	408	Heikkilä et al. 2015	Suture on metascutum below where microtrichi	absent (0)	present (1)	present and fused to a
	56	388	Heikkilä et al. 2015	Fenestra media of metapostnotum	small not expanded (0)	large expanded (1)	
	57	389	Heikkilä et al. 2015	Metathorax fenestrae laterales	absent or reduced (0)	present (1)	
	58	8	Sharplin 1964	Metabasalare	Second basalare of the hind wing	Second basalare free, but hav	Typical basalare of hig
	59	12	Sharplin 1964	Insertion of IIItpm2	arising on the pleural ridge and ir	inserting on the metaprescuti	inserting on the poste
	60	390	Heikkilä et al. 2015	Metathoracic tympanal organs	absent (0)	present (1)	
	61	409	Heikkilä et al. 2015	Metathorax coxal process	absent (0)	present (1)	
	62	410	Heikkilä et al. 2015	Metacoxal trochantin elongated	no (0)	yes (1)	
	63	6	Sharplin 1964	Median plates	Median plates present in the hin	Median plates replaced in the hind wing by cubital p	
	64	7	Sharplin 1964	Median plates	Media connected to the distal me	The base of the media, when present in the hind wi	
	65	405	Heikkilä et al. 2015	Metathoracic furca with ventrodistal extension	no (0)	yes (1)	
	66	406	Heikkilä et al. 2015	Internal laminae of secondary arm of metafurca	absent (0)	present (1)	
	67	413	Heikkilä et al. 2015	Posterior tendons of metafurcal apohyses elong	absent (0)	present (1)	
	68	414	Heikkilä et al. 2015	Furcal bridge fusion of metafurca to the second	absent (0)	present (1)	
	69	415	Heikkilä et al. 2015	Laminae between posterior tendons and second	absent (0)	present (1)	
	70			IIIdlm2	absent (0)	present (1)	
	71			IIIdlm3	absent (0)	present (1)	
	72			IIIdvm1	absent (0)	present (1)	
	73			IIIdvm8	absent (0)	present (1)	
	74			IIItpm1	absent (0)	present (1)	
	75			IIItpm2	absent (0)	present (1)	
	76			IIItpm3	absent (0)	present (1)	
	77			IIItpm4	absent (0)	present (1)	
	78			IIItpm5	absent (0)	present (1)	
	79			IIItpm10	absent (0)	present (1)	
	80			IIIspm1	absent (0)	present (1)	
	81			IIIspm2	absent (0)	present (1)	
	82			IIIpcm2	absent (0)	present (1)	
	83			IIIpcm4	absent (0)	present (1)	
	84			IIIvlm2	absent (0)	present (1)	
	85			IIIscm1	absent (0)	present (1)	
Pterothorax	**86**	1	Sharplin 1964	Tergopleural apodeme	absent (0/-)	the tergopleural apodeme is f	the tergopleural apod
	87	13	Sharplin 1964	Anal plate	present (0/S)	absent (1/A)	

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3 **4** **5**

bridge (1)

: inflated structure with connecting part extending far down along sides of pronotum (2)
gia (1)

ural hind margin (1)
relatively close clefts continue as sutures that meet medially (3)
2)

› the anapleural cleft (1)
zed depression (2)

ral suture (1)
ving process and first axillary sclerite, between 1/50 and 1/20 of the wing length (2/A)
resent (2/A)
Large, regular cone-shaped pegs only. (3/A)
ent in the mesothorax. (1/A)

apex strengthen and articulating with pro-spinasternum (2)

ed with the second basalare (1/A)

anteriorly directed sutures of metascutellum (2)

gher Lepidoptera (2/A)
erior side of laterophragma of the mesothorax (2/A)

plates (1/A)
ing, not reaching the median arm or median plates (1/A)

tergopleural and prescutal apodemes fused (3/A)

idoptera). *BMC Evolutionary Biology, 15*, 260.
2.

Study I

Appendix 4: Character matrix

| Source | Name | | |

| | | |

Heikkilä et al. 2015; **Arctiini_Erebidae**	Heikkilä et al. 2015; **Plusiinae_Noctuidae**	Bharadwaj et al. 197;**Hadeninae_Noctuidae**	Heikkilä et al. 2015; **Heliothinae_Noctuidae**
2	2	2	2
0	0	0	0
1	1	1	1
0	0	0	0
0	0	0	0
1	1	1	1
0	0	0	0
1	1	0	0
1	1	1	1
1	1	1	1
1	1	1	1
0	0	0	0
1	?	1	1
0	0	0	0
1	1	1	1
0	0	0	0
1	1	1	1
0	0	0	0
1	?	2	2
0	0	0	0
1	1	1	1
2	2	2	2
2	2	2	2
3	3	3	3
1	1	1	1
1	1	1	1
0	0	0	0
2	2	2	2
1	1	1	1
0	0	0	0
0	0	0	0
1	1	1	1
0	0	0	0
0	0	0	0
1	1	1	1
0	1	1	0
0	0	0	0
1	0	1	1
1	1	0	1
1	1	0	0
1	0	0	0
0	0	0	0
1	1	1	1
0	0	0	0
1	1	1	1
0	0	0	0
1	1	1	1
1	1	1	1
1	1	1	1
1	1	1	1
1	1	1	1
1	1	1	1
1	1	1	1
0	0	0	0
0	0	0	0
1	1	1	1
2	2	2	2
2	2	2	2
1	1	1	1
0	0	0	0
0	0	0	0
1	1	1	1
1	1	1	1
0	0	0	0
1	1	1	1
0	0	0	0
0	0	0	0
?	?	?	?

0 1 1 1
0 0 0 0
1 1 1 1
0 0 0 0
0 1 0 0
1 0 0 1
0 0 0 0
1 1 0 1
0 0 0 0
0 0 0 0
1 1 1 1
1 1 1 1
1 1 1 1
1 1 1 1
1 1 1 1
1 1 1 1
3 3 3 3
(0, (0, (0, (0,1)

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Study III

Appendix 1: Diptera muscular homology chart

Reviewed and published versions are same.

Muscle present is represented by "+" in green, absent by "-" in pink, uncertain by "?" or "/" in yellow.

Family	Tipulidae		Culicidae		Anisopodidae	Tabanidae	Stratiomyidae	Dolichopodidae & Empididae	Microphoridae	Syrphidae	Micropezidae
Species	*C. mikado*	*T. vernalis*	*A. aegypti*	*C. inornata*	*Anisopus*	*T. sulcifrons*	*P. latifascia*		*M. holosericeus*	*L. obscuritarsis*	*C. sinensis*
References	Maki 1938	Mickoleit 1962	Christophers 1960	Owen 1977	Smart 1959	Bonhag 1949	Maki 1938	Ulrich 1971	Ulrich 1984	Maki 1938	Maki 1938
Idlm1	-	-	29&30	1&2	/	1	-	-	-	-	-
Idlm2	-	-	-	-	/	2	2&3	2	2&3	2	2
Idlm3	1	1	35&36&37?	3	/	-	1	1&3	1	1	1
Idlm4	-	-	31	-	/	-	-	-	-	-	-
Idlm5	-	-	-	-	/	-	-	-	-	3	3
Idlm6	-	-	-	-	/	-	-	-	-	-	-
Idvm1	6&7?	2?	27&28?	4&5?	/	3	-	4?	4?	7?&8?&9?&10?	-
Idvm2	6&7?	2?	27&28?	4&5?	/	4?	-	4?	4?	7?&8?&9?&10?	-
Idvm3	6&7?	2?	27&28?	4&5?	/	4?	-	4?	4?	7?&8?&9?&10?	-
Idvm4	8	-	34	-	/	-	6	-	-	-	9&10
Idvm5	-	-	38?	10?	/	5	7&9	6	6	12	12
Idvm6	-	-	-	-	/	-	-	-	-	-	-
Idvm7	-	-	-	-	/	8&9	8&10	5	5&7	11	11
Idvm8	-	-	-	-	/	-	-	-	-	-	-
Idvm9	-	-	-	-	/	-	-	-	-	-	-
Idvm10	-	-	-	-	/	-	-	-	-	-	-
Idvm11	-	-	-	-	/	-	-	-	-	-	-
Idvm12	9	-	-	-	/	-	-	-	-	-	-
Idvm13	-	-	-	-	/	-	-	-	-	-	-
Idvm14	-	-	-	-	/	-	-	-	-	-	-
Idvm15	-	-	-	-	/	-	-	-	-	-	-
Idvm16	-	-	-	-	/	-	-	-	-	-	-
Idvm17	-	-	-	-	/	-	-	-	-	-	-
Idvm18	-	-	-	-	/	-	-	-	-	-	15?
Idvm19	-	-	63	11	/	-	16	-	-	19	-
Itpm1	-	-	-	-	/	-	-	-	-	-	-
Itpm2	-	-	-	-	/	-	-	-	-	-	-
Itpm3	9	16	-	-	/	-	-	-	-	-	-
Itpm4	-	-	-	-	/	-	-	-	-	-	-
Itpm5	-	-	-	-	/	-	-	-	-	-	-
Itpm6	-	-	58	21	/	-	-	-	-	-	-
Ippm1	-	3?	33?	-	/	-	-	7?	-	-	-
Ippm2	-	3?	33?	-	/	-	-	7?	-	-	-
Ispm1	-	-	-	-	/	-	-	-	-	-	-
Ispm2	-	-	-	-	/	-	-	-	-	-	-
Ispm3	-	-	-	-	/	-	-	-	-	-	-
Ispm4	-	-	-	-	/	-	-	-	-	-	-
Ispm5	-	-	-	-	/	-	-	-	-	-	-
Ispm6	-	-	-	-	/	-	-	-	-	-	-
Ispm7	-	-	-	-	/	-	-	-	-	-	-
Ipcm1	-	-	32	8&9	/	17	11	11	11	13&14	13
Ipcm2	-	-	-	-	/	-	-	-	-	-	-
Ipcm3	-	-	-	-	/	-	-	-	-	-	-
Ipcm4	13&14	9	-	14	/	16	14&15	14	14	18	18
Ipcm5	-	-	69	-	/	-	-	-	-	-	-
Ipcm6	-	-	-	-	/	15	-	-	13a, d	-	-
Ipcm7	-	-	-	-	/	-	-	-	-	-	-
Ipcm8	15&16	10&11	62	12	/	19a	17	16a	16a	-	19
Ivlm1	3&4	5&17	-	-	/	6&7	5	9&10	9&10	5&6	7&8
Ivlm2	-	-	-	-	/	-	-	-	-	-	-
Ivlm3	2	4	25?&26?	6&7	/	10&11	4	8	8	4	4&5&6
Ivlm4	-	-	-	-	/	-	-	-	-	-	-
Ivlm5	-	-	-	-	/	-	-	-	-	-	-
Ivlm6	-	-	-	-	/	-	-	-	-	-	-
Ivlm7	-	-	-	-	/	-	-	-	-	-	-
Ivlm8	-	-	-	-	/	-	-	-	-	-	-
Ivlm9	-	-	-	-	/	-	-	-	-	-	-
Iscm1	11	6	70	15	/	12	12	12	12	15&16	14
Iscm2	12	7	71	16	/	13	13	13	13b, c	17	16&17
Iscm3	-	8	-	-	/	14	-	15	15	-	-
Iscm4	-	-	-	-	/	-	-	-	-	-	-
Iscm5	-	-	-	-	/	-	-	-	-	-	-
Iscm6	17	12	64	13	/	19b	18	16b	16b	20	-
Iscm7	-	-	-	-	/	-	-	-	-	-	-
IIdlm1	18	29	39	47&48	1	27	19	17	17	21	20
IIdlm2	19	30	39	-	2	28	20	18	18	22	21
IIdlm3	-	-	43	55	-	-	-	-	53	-	-
IIdvm1	22	27	41	49&50&51	4	29	21	19	19	23	22
IIdvm2	-	-	-	-	-	-	-	-	-	-	-
IIdvm3	-	-	-	-	-	-	-	-	-	-	-
IIdvm4	36?	28?	41	52	5	30?	36?	30?	30?	38?	37?
IIdvm5	36?	28?	42	53	5	30?	36?	30?	30?	38?	37?
IIdvm6	37	32	?	57&58	10	- (39)	-	-	-	-	-
IIdvm7	-	-	-	-	- (6)	46a	38&39	33a	33a	40&41	39&40
IIdvm8	-	-	-	-	-	-	-	-	-	-	-
IIdvm9	-	-	-	-	-	-	-	-	-	-	-
IItpm1	24	44	44	64	8a	31	23	20	20	25	24
IItpm2	25	42	45	56	7b	42	24	22	22	26	25
IItpm3	23	45&46	46	65	7a	32	22	21	21	24	23
IItpm4	26&27	33&34	47	63	9a	34	25&26	24	24	27&28	26&27
IItpm5	-	-	-	-	-	-	-	-	-	-	-
IItpm6	30&31	38&39&40&43	50?&52?&53?	59&60	7c&9c	36&37&38	29&30&31	23	23	32&33	30&31&32
IItpm7	28	35	49	62	9bi	35a	27	25a	25a	29	28
IItpm8	-	-	-	-	-	-	-	-	-	-	-
IItpm9	29	36&37	48	61	9bii	35b	28	25b	25b	30&31	29
IItpm10	-	-	-	-	-	-	-	-	-	-	-
IItpm11	-	-	-	-	-	-	-	-	-	-	-
IItpm12	-	-	-	-	-	-	-	-	-	-	-
IIppm1	-	-	-	-	-	-	-	-	-	-	-
IIppm2	-	-	-	-	-	-	-	-	-	-	-
IIspm1	32	31	59	22	8b	33	32	26	26	34	33
IIspm2	33	47&48	54	25	11	40&41	33&34	27&28	27&28	35&36	34&35
IIspm3	-	-	-	-	-	-	-	-	-	-	-
IIspm4	-	-	-	-	-	-	-	-	-	-	-
IIspm5	-	-	-	-	-	-	-	-	-	-	-
IIspm6	-	-	-	-	-	-	-	-	-	-	-
IIspm7	-	-	-	-	-	-	-	-	-	-	-

IIspm8	-	-	-	-	-	-	-	-	-	-	-
IIpcm1	-	-	-	-	-	-	-	-	-	-	-
IIpcm2	-	-	-	-	-	-	-	-	-	-	-
IIpcm3	-	-	-	-	-	-	-	-	-	-	-
IIpcm4	-	-	-	-	-	-	-	-	-	-	-
IIpcm5	-	22	72	23	- (8c)	-	-	-	-	-	-
IIpcm6	39	24	-	-	13a	47a	40	34	34	41	41
IIvlm1	-	-	-	-	-	-	-	-	-	-	-
IIvlm2	-	-	-	-	-	-	-	-	-	-	-
IIvlm3	44	41	-	-	- (3)	43	43&44	29	29	45	44
IIvlm4	-	-	-	-	-	-	-	-	-	-	-
IIvlm5	-	-	-	-	-	-	-	-	-	-	-
IIvlm6	-	-	-	-	-	-	-	-	-	-	-
IIvlm7	-	-	-	-	-	-	-	-	-	-	-
IIscm1	35	18	78	28	14a	45	35	31	31	37	36
IIscm2	38	19	79	26&27&29	-	44	37	32	32	39	38
IIscm3	-	-	-	-	-	-	-	-	-	-	-
IIscm4	34	20	-	-	-	-	-	-	-	-	-
IIscm5	-	-	-	-	14b	-	-	-	-	-	-
IIscm6	-	21	73	24	12	46b	-	33b	33b	-	-
IIscm7	-	-	-	-	-	-	-	-	-	-	-
IIIdlm1	43	-	40	54	/	-	42	-	36	44	43
IIIdlm2	-	-	-	-	/	-	-	-	-	-	-
IIIdlm3	-	-	-	-	/	-	-	-	-	-	-
IIIdvm1	46	60	-	-	/	56	45	37	37	46	45
IIIdvm2	-	-	-	-	/	-	-	-	-	-	-
IIIdvm3	-	-	-	-	/	-	-	-	-	-	-
IIIdvm4	-	-	-	-	/	-	-	-	-	-	-
IIIdvm5	-	-	-	-	/	-	-	-	-	-	-
IIIdvm6	54	-	-	-	/	-	-	-	-	-	-
IIIdvm7	-	54	-	-	/	69a	55	50a	50a	-	56
IIIdvm8	-	-	-	-	/	-	-	-	-	-	-
IIItpm1	-	64	-	-	/	59	46	38	38	47	46
IIItpm2	-	-	-	-	/	-	-	36	-	-	-
IIItpm3	47	-	-	-	/	-	-	-	-	-	-
IIItpm4	-	-	-	-	/	62	47	39	39	48	47
IIItpm5	-	-	-	-	/	-	-	-	-	-	-
IIItpm6	48	65	55	45	/	-	-	40	40	-	-
IIItpm7	-	-	?	46	/	-	-	-	-	-	-
IIItpm8	-	-	-	-	/	-	-	-	-	-	-
IIItpm9	49	63	56?	44?	/	60	48	41	41	49	48
IIItpm10	-	-	-	-	/	-	-	-	-	-	-
IIItpm11	-	62	-	-	/	61	49	43	43	50	49
IIItpm12	-	-	-	-	/	-	-	-	-	-	-
IIIppm1	-	-	-	-	/	-	-	-	-	-	-
IIIppm2	-	-	-	-	/	-	-	-	-	-	-
IIIspm1	50	61	-	-	/	57	50	42a	42a	51	50
IIIspm2	51&52	66&67	-	-	/	63&64	51&52	44&45	44&45	52&53	51&52
IIIspm3	-	-	-	-	/	-	-	-	-	-	-
IIIspm4	-	-	-	-	/	-	-	-	-	-	-
IIIspm5	-	-	-	-	/	-	-	-	-	-	-
IIIpcm1	-	-	-	-	/	-	-	-	-	-	-
IIIpcm2	56	52	-	-	/	58&66	-	42b	42b	56	55
IIIpcm3	-	-	-	-	/	-	-	-	-	-	-
IIIpcm4	-	-	-	-	/	65	-	49	49	-	-
IIIpcm5	57	55	80	34	/	-	-	-	-	-	-
IIIpcm6	58&59&61	57	-	-	/	72a	58	51	51	58	58
IIIpcm7	-	-	-	-	/	-	-	-	-	-	-
IIIvlm1	-	-	-	-	/	-	-	-	-	-	-
IIIvlm2	+	71	?	42	/	73	+	46	46	+	+
IIIvlm3	-	-	-	-	/	-	-	-	-	-	-
IIIscm1	53	49	86	36	/	67	53	47	47	54	53
IIIscm2	55	50	87	37	/	68	54	48	48	55	54
IIIscm3	-	-	-	-	/	-	-	-	48?	-	-
IIIscm4	-	51	-	-	/	-	-	-	-	-	-
IIIscm5	-	-	-	-	/	-	-	-	-	-	-
IIIscm6	60	53	81	35	/	69b	56&57	50b	50b	57	57

Drosophilidae	Glossinidae	Nycteribiidae		Streblidae	Hippoboscidae						Muscidae	
D. melanogaster	*G. longipalpis*	*B. nana*		*N. alluaudi*	*P. canariensis*	*H. longipennis*	*L. capreoli*	*M. ovinus*	*S. hirundinis*	*C. pallida*	*M. domestica*	*O. claripennis*
Fabian et al. 2016	Schlein 1970	Nußbaum 1960	Sclein 1970	Schlein 1970						present study	Schlein 1970	Maki 1938
+	A1	1	-	A1?&A2	A2	A2	A2	A2	A2	+	A1&A2	A1
+	A2	-	A2	-	-	-	-	-	-	-	A2	A2&A3
-	-	5	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	A3	-	A3?	A3	A3	A3	A3	A3	A3	+	A3	A9&A10?
+	A4	-	A3?	A4?	A4?	A4?	A4?	A4?	A4?	+	A4	A9&A10?
+	A4	-	A3?	A4?	A4?	A4?	A4?	A4?	A4?	+	A4	A9&A10?
+?	-	2	-	-	-	-	-	-	-	-	-	-
-	A5&A9	-	A5	A5	A5&A9	A5&A10	A5&A11	A5&A12	A5&A13	+	A5&A9	A12
-	-	-	-	-	-	-	-	-	-	-	-	-
-	A5&A9	-	-	-	A5?&A9?	A5?&A9?	A5?&A8&A9?	A5?&A8&A9?	A5?&A9?	+	A5&A9	A11
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	7&10&13	-	-	-	-	-	-	-	+	-	-
-	-	8	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	9	A15	A15	A15	A15	A15	A15	A15	+	A15	A15?
+	A17	-	A17	A17	A17	A17	A17	A17	A17	+	A17	A18
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	39	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	A16	3	A16	-	A16	A16	-	-	A16	-	A16	13
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	17
-	-	-	-	-	-	-	-	-	-	-	-	-
-	A15	-	-	A15	-	-	-	-	-	-	A15	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	A6&A7&A8	-	-	A6	A6	A6	A6	A6	A6	+	A6&A7&A8	7&8
-	-	-	-	-	-	-	-	-	-	-	-	-
+	A10	4	A10	A10	A10	A10	A10	A10	A10	+	A10&A11	4&5&6
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	+	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	A12	6	A12	A12	A12	A12	A12	A12	A12	+	A12	14
-	A13	11	A13	A13	A13	A13	A13	A13	A13	+	A13	16
-	A14	14	A14	A14	A14	A14	A14	A14	A14	+	A14	-
-	-	12	-	-	-	-	-	-	-	+	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	A17	-	A17	A17	A17	A17	A17	A17	A17	+	A17	19
-	-	-	-	-	-	-	-	-	-	-	-	-
+	?	-	?	?	?	?	?	B1	B1	+	?	20&21
+	B2	40?	-	B2	B2	B2	B2	B2	B2	+	B2	22
-	-	-	-	-	-	-	-	-	-	-	-	-
-	B3	-	B3	B3	B3	B3	B3	B3	B3	-	B3	23
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+?	B4?	B4?	B4?	B4?	B4?	B4?	B4?	B4?	B4?	-	B4?	40?
+?	B4?	B4?	B4?	B4?	B4?	B4?	B4?	B4?	B4?	-	B4?	40?
-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	21b	B19	B19	-	-	-	-	-	-	B19	42&43
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	B5	-	-	B5	B5	B5	B5	B5	B5	+	B5	25
-	B15	-	?	B15	B15	B15	B15	B15	B15	-	B15	26
+	-	-	-	-	-	-	-	-	-	-	B6	24
+	B8	-	-	B8	B8	B8	B8	B8	B8	+	B8	27&28
-	-	-	-	-	-	-	-	-	-	-	-	-
+	B10&B12	-	-	B10&B12	B10&B12	B10&B12	B10&B12	B10&B12	B10&B12	+	B10&B12	31&32&33&34
+	-	-	-	B9	B9	B9	B9	B9	B9	+	B9	29
-	-	-	-	-	-	-	-	-	-	-	-	-
+	B9	-	-	?	?	?	?	?	?	+	B9	30
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	B7	-	-	B7	B7	B7	B7	B7	B7	-	B7	35
+	B13&B14	-	-	B13	B13&B14	B13&B14	B13	B13	B13	+	B13&B14	36&37
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-

-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	+	-	-
-	-	21a	-	-	-	-	-	-	-	-	-	-
+	B20	25	B20	B20	B20	B20	B20	B20	B20	+	B20	44
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	B16	-	-	B16	B16	B16	B16	B16	B16	+	B16	47
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	B18	19	B18	B18	B18	B18	B18	B18	B18	+	B18	38&39
+	B17	20	B17	B17	B17	B17	B17	B17	B17	+	B17	41
-	-	-	-	-	-	-	-	-	-	+	-	-
+	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	B19	22	B19	B19	B19	B19	B19	B19	B19	+	B19	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	46
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	C1	38	?	C1	-	C1	-	-	C1	-	C1	48
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	C14	-	?	-	-	-	-	-	-	-	C14	59
-	-	-	-	-	-	-	-	-	-	-	-	-
-	C4	-	-	-	-	-	-	-	-	-	C4	49
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+?	-	-	-	-	-	-	-	-	-	+	-	50
+?	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	C5	37	?	C5	C5	C5	C5	C5	C5	+	C5	51
+?	-	-	-	-	-	-	-	-	-	-	-	-
-	C6	-	?	?	C6	C6	C6	C6	C6	-	C6	52
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+?	-	-	-	-	-	-	-	-	-	-	-	-
+	C2	-	-	-	-	-	-	-	-	-	C2	53
+	C8&C9	-	C8	C8	-	C8	C8	C8	C8	-	C8&C9	54&55
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	C3&C11	29	C11	C3&C11	C3&C11	C3&C11	C3&C11	C3&C11	C3&C11	+	C3&C11	58
-	-	-	-	-	-	-	-	-	-	-	-	-
+	C10	-	C10	C10	C10	C10	C10	C10	C10	-	C10	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	C15	-	C15	C15	C15	C15	C15	C15	C15	-	C15	61
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	-	-	-	-	-	-	-	-	-	-	C16	+
-	-	-	-	-	-	-	-	-	-	-	-	-
+	C12	28	C12	-	C12	C12	C12	C12	C12	+	C12	56
+	C13	30	C13	C13	C13	C13	C13	C13	C13	+	C13	57
-	-	-	-	-	-	-	-	-	-	+	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-
+	C14	31	?	C14	C14	C14	C14	C14	C14	+	C14	60

Study IV

Appendix 1: Combined skeletomuscular characters (Reviewed Version)

References	Baroni Urbani 1992 (BU92)																							
Original No.	10	11	12	13	14	15	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
No.					1		2	3		4	5			6	7	8	9	10	11	12		13	14	
Myrmicinae	1	1	0	1	0	0	2	1	0	2	1	0	0	0	2	1	1	1	1	0	0	1	1	(0,1)
Ectatomminae	?	?	?	?	?	?	1	1	0	0	0	0	(0,1)	0	1	0	1	1	1	0	0	1	1	0
Heteroponerinae	?	?	?	?	?	?	0	0	0	1	1	0	1	0	1	(0,1)	1	1	0	1	0	1	1	0
Formicinae	(0,1)	(0,1)	0	1	0	0	0	0	0	0	(0,1)	0	0	0	0	0	0	(0,1)	0	0	0	1	1	0
Pseudomyrmicinae	0	1	1	1	0	(0,1)	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
Myrmeciinae	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	?	0	0	0	0
Dolichoderinae	0	1	0	1	0	0	0	0	0	0	1	0	0	0	(0,1)	0	1	1	0	0	0	1	1	0
Aneuretinae	0	1	0	(0,1)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1
Aenictinae	1	1	1	1	1	0	2	2	0	2	1	0	0	1	2	1	1	1	0	2	0	1	1	0
Dorylinae	1	1	1	1	1	0	1	1	0	2	1	0	0	0	2	1	2	1	0	2	0	1	1	0
Cerapachyinae	(0,1)	1	1	1	(0,1)	0	(1,2)	1	0	2	1	0	(0,1)	(0,1)	2	(0,1)	1	1	0	2	0	1	1	0
Ecitoninae	1	1	1	1	1	0	2	2	0	2	1	0	0	1	2	1	1	1	0	2	0	(0,1)	(0,1)	0
Leptenilloidinae	0	1	?	1	0	0	0	0	0	2	1	0	0	0	2	1	2	1	0	2	0	1	1	0
Ponerinae	(0,1)	1	0	(0,1)	0	(0,1)	0	0	0	2	1	0	0	0	2	0	1	1	0	0	0	1	1	0
Agroecomyrmecinae	?	?	?	?	?	?	2	1	0	2	1	0	0	0	2	1	1	1	1	0	0	1	1	0
Paraponerinae	?	?	?	?	?	?	1	1	0	2	1	0	0	0	1	0	1	1	0	0	0	1	0	0
Proceratiinae	?	?	?	?	?	?	2	(1,2)	0	2	1	0	0	1	2	1	1	1	0	0	0	1	1	0
Amblyoponinae	0	1	0	1	0	0	0	0	0	1	0	0	0	0	1	1	1	1	2	0	0	1	1	0
Leptanillinae	0	1	1	1	0	0	0	0	0	2	1	0	0	0	2	1	1	1	0	1	0	1	1	0
Scoliidae	?	?	?	?	?	?	0	0	0	-	-	0	0	0	-	0	-	0	-	-	0	0	0	0
Bradynobaenidae	0	0	0	(0,1)	0	0	0	?	?	?	?	?	?	?	?	?	?	0	?	0	?	?	?	?
Vespidae	0	0	0	(0,1)	0	0	0	0	0	-	-	0	0	0	-	0	-	0	-	0	0	0	0	0

Used Characters		
BU92: 14	1	Worker. 'Metatibial gland' present (1), or absent (0)
K11: 49 (BU92: 10)	2	Promesonotal junction: (0) articulated; (1) sutured; (2) fused, indistinct.
K11: 50	3	Pronotomesepisternal junction: (0) articulated; (1) sutured; (2) fused, indistinct.
K11: 52	4	Mesonotum: (0) round and dome shaped; (1) longitudinally narrow and flat; (2) continuous or near continuous with metanotum and propodeum in profil
K11: 53	5	Notopleural suture on mesothorax: (0) present; (1) absent.
K11: 56	6	Mesothoracic spiracle: (0) concealed by a spiracular lobe; (1) exposed.
K11: 57	7	Metanotum: (0) discernable on dorsal mesosoma as a transverse striplike sclerite; (1) present only as a groove; (2) obliterated.
K11: 58	8	Metathoracic spiracle: (0) concealed by spiracular lobe; (1) exposed, opening round to oval.
K11: 59	9	Metathoracic spiracle orientation: (0) dorsad; (1) posterolaterad; (2) anterolaterad.
K11: 60 (BU92: 11)	10	Metapleural gland: (0) absent; (1) present.
K11: 61	11	Ventral flap on metapleural gland opening: (0) absent; (1) bulla shaped and unarmed; (2) oblong and armed with a keellike projection.
K11: 62 (BU92: 12)	12	Metapleural longitudinal flange: (0) absent; (1) projecting laterad; (2) projecting ventrolaterad, concealing the metapleural gland opening.
K11: 64	13	Propodeal spiracle: (0) with bulla; (1) without bulla.
K11: 65	14	Propodeal spiracle atrial opening: (0) slit shaped; (1) round to oval.
K11: 67	15	Propodeal lobe: (0) absent; (1) present.
K11: 69	16	Metacoxal cavity: (0) open; (1) closed.
K11: 71	17	Stout setae on posterior apex of protibia: (0) absent; (1) one; (2) two.
K11: 72	18	Stout setae on posterior surface of probasitarsal noth: (0) absent; (1) single; (2) row parallel to strigil comb.
K11: 74	19	Calcar of strigil: (0) fully pectinated; (1) with a basal lamella; (2) fully lamellated.
K11: 76	20	Brush on posterior surface of strigil calcar: (0) absent; (1) present.
K11: 78	21	Squamiform brush on calcar anterior surface of strigil: (0) absent; (1) present.
K11: 79	22	Setae on anterior surface of probasitarsal notch: (0) absent; (1) paddle shaped; (2) serrated.
K11: 80	23	Protarsus fourth tarsomere: (0) with lateral lobate projections; (1) conical; (2) cylindrical.
K11: 82	24	Claws in propretarsus: (0) simple; (1) with preapical tooth; (2) pectinate; (3) with basal spines.
K11: 83	25	Arolium in propretarsus: (0) vestigial to absent; (1) present.
K11: 84	26	Arolium in mesopretarsus and metapretarsus: (0) vestigial to absent; (1) present.
K11: 88	27	Anterior metatibial spur: (0) absent; (1) simple; (2) pectinate.
K11: 89	28	Posterior metatibial spur: (0) absent; (1) simple; (2) pectinate.
K11: 91	29	Mesobasitarsal sulcus: (0) absent; (1) present.
K11: 92	30	Metabasitarsal sulcus: (0) absent; (1) present.

Unused Characters		
BU92: 10	1	Worker. Promesonotal suture fused (1) or mobile (0).
BU92: 11	2	Worker and gyne. Metapleural gland present (1) or absent (0).
BU92: 12	3	Worker and gyne. Opening of the metapleural gland covered from above by a cuticular flange (1), or not (0).
BU92: 13	4	Worker, gyne and male. Metacoxal cavities open or sutured (0), or closed (1).
BU92: 15	5	Worker and gyne. Basitarsal sulcus on metatarsi present (1) or absent (0).
K11: 51	6	Stout, peglike setae on pronotal disc: (0) absent; (1) present.
K11: 54	7	Mesonotal lateral margins: (0) parallel to subparallel; (1) posteriorly tapering, dorsum triangular.
K11: 55	8	Median sulcus on mesepisterna: (0) absent; (1) present.
K11: 63	9	Propodeal lateral margins: (0) parallel to subparallel; (1) upper anterior part strongly constricted.
K11: 66	10	Propodeal spines: (0) absent; (1) present.
K11: 68	11	Propodeal foramen: (0) free and visible; (1) enclosed in tubular projection.
K11: 70	12	Protibial anterior sulcus: (0) absent; (1) present.
K11: 73	13	Basal projection on inner portion of probasitarsus: (0) absent; (1) rounded; (2) acute.
K11: 75	14	Lamella of strigil calcar: (0) entire; (1) with a notch.
K11: 77	15	Longitudinal sulcus on probasitarsal posterior surface: (0) absent; (1) present.
K11: 81	16	Propretarsal manubrium: (0) flat and discoidal; (1) protruded distad.
K11: 85	17	Spinelike setae on midleg: (0) absent; (1) present on tibia and basitarsus; (2) present on basitarsus only.
K11: 86	18	Spinelike setae on metabasitarsus: (0) absent; (1) present.
K11: 87	19	Metacoxal dorsum: (0) unarmed; (1) armed with a tooth or spine.
K11: 90	20	Microtrichia on metatibial spur posterior face: (0) absent; (1) present, simple; (2) present, antlerlike.
K11: 93	21	Metabasitarsal ventral longitudinal groove: (0) absent; (1) present.
K11: 94	22	Basitarsal gland on metatarsus: (0) absent; (1) present.

Keller 2011 (K11)																											
67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94
15		16		17	18		19		20		21	22	23		24	25	26				27	28		29	30		
1	0	1	0	1	(0,1)	0	0	0	0	0	(0,1)	1	1	0	0	1	1	0	0	0	0	2	0	0	0	0	0
1	0	0	0	1	2	0	(0,1)	0	0	0	1	1	1	0	1	0	0	0	0	0	0	(1,2)	0	0	0	0	0
1	0	1	0	1	(1,2)	0	1	0	1	0	1	1	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0
(0,1)	0	1	0	0	(0,1)	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
1	0	1	0	0	(0,2)	0	1	0	1	0	1	1	0	0	1	1	1	0	0	0	1	2	0	(0,1)	1	0	0
1	0	0	0	(1,2)	(1,2)	0	1	0	0	0	1	1	0	0	1	1	1	0	0	0	(1,2)	2	0	1	1	0	0
0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	2	0	0	0	0	0
1	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0
1	0	1	0	0	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0
0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	2	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0	0	(1,2)	(0,1)	0	(0,1)	1	1	(0,1)	0	0	(0,2)	2	0	0	0	(0,1)	0
1	0	1	0	1	(0,1)	0	0	0	0	0	0	2	(0,1)	0	1	1	(0,1)	0	0	0	0	2	0	0	0	0	0
1	1	1	0	0	0	0	0	0	0	0	0	2	1	0	0	1	1	0	0	0	0	2	0	0	0	0	0
1	0	0	0	(0,1)	(1,2)	0	1	0	(0,1)	0	1	1	0	0	1	1	1	0	0	0	(1,2)	2	1	0	0	0	0
1	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	2	0	0	0	0	0
1	0	0	0	0	2	0	1	0	1	1	1	1	0	0	1	1	1	0	0	0	0	2	0	1	1	0	0
1	0	1	0	0	0	0	0	0	0	0	1	1	2	0	0	1	1	0	0	0	0	2	0	0	0	0	0
1	0	0	0	(0,1)	0	0	(0,1)	0	0	0	1	1	1	0	0	1	1	0	0	0	(0,1)	2	0	0	0	0	(0,1)
0	0	1	0	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	0	0	1	2	0	0	0	0	0
1	0	?	0	2	0	0	2	0	0	0	0	0	0	0	0	1	1	0	0	0	2	2	0	0	0	0	0
?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
1	0	1	0	2	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	2	2	0	0	0	0	0

e.

Study IV

Appendix 1: Character list and matrix (Published Version)

References	Baroni Urbani 1992 (BU92)																							
Original No.	10	11	12	13	14	15	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
No.							1	2		3	4				5	6	7	8	9	10		11	12	
Myrmicinae	1	1	0	1	0	0	2	1	0	2	1	0	0	0	2	1	1	1	1	0	0	1	1	(0,1)
Ectatomminae	?	?	?	?	?	?	1	1	0	0	0	0	(0,1)	0	1	0	1	1	1	0	0	1	1	0
Heteroponerinae	?	?	?	?	?	?	0	0	0	1	1	0	1	0	1	(0,1)	1	1	0	1	0	1	1	0
Formicinae	(0,1)	(0,1)	0	1	0	0	0	0	0	0	(0,1)	0	0	0	0	0	0	(0,1)	0	0	0	1	1	0
Pseudomyrmecinae	0	1	1	1	0	(0,1)	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
Myrmeciinae	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	?	0	0	0	0
Dolichoderinae	0	1	0	1	0	0	0	0	0	0	1	0	0	0	(0,1)	0	1	1	0	0	0	1	1	0
Aneuretinae	0	1	0	(0,1)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1
Dorylinae	1	1	1	1	1	0	2	(0,1,2)	0	2	1	0	0	(0,1)	2	1	1	1	0	2	0	1	1	0
Ponerinae	(0,1)	1	0	(0,1)	0	(0,1)	0	0	0	2	1	0	0	0	2	0	1	1	0	0	0	1	1	0
Agroecomyrmecinae	?	?	?	?	?	?	2	1	0	2	1	0	0	0	2	1	1	1	1	0	0	1	1	0
Paraponerinae	?	?	?	?	?	?	1	1	0	2	1	0	0	0	1	0	1	1	0	0	0	1	0	0
Proceratiinae	?	?	?	?	?	?	2	(1,2)	0	2	1	0	0	1	2	1	1	1	0	0	0	1	1	0
Amblyoponinae	0	1	0	1	0	0	0	0	0	1	0	0	0	0	1	1	1	1	2	0	0	1	1	0
Leptanillinae	0	1	1	1	0	0	0	0	0	2	1	0	0	0	2	1	1	1	0	1	0	1	1	0
Scoliidae	?	?	?	?	?	?	0	0	0	-	-	0	0	0	-	0	-	0	-	-	0	0	0	0
Bradynobaenidae	0	0	0	(0,1)	0	0	0	?	?	?	?	?	?	?	?	?	?	0	?	0	?	?	?	?
Vespidae	0	0	0	(0,1)	0	0	0	0	0	-	-	0	0	0	-	0	-	0	-	0	0	0	0	0

Used Characters		
K11: 49 (BU92: 10)	1	Promesonotal junction: (0) articulated; (1) sutured; (2) fused, indistinct.
K11: 50	2	Pronotomesepisternal junction: (0) articulated; (1) sutured; (2) fused, indistinct.
K11: 52	3	Mesonotum: (0) round and dome shaped; (1) longitudinally narrow and flat; (2) continuous or near continuous with metanotum and propodeum in profil
K11: 53	4	Notopleural suture on mesothorax: (0) present; (1) absent.
K11: 57	5	Metanotum: (0) discernable on dorsal mesosoma as a transverse striplike sclerite; (1) present only as a groove; (2) obliterated.
K11: 58	6	Metathoracic spiracle: (0) concealed by spiracular lobe; (1) exposed, opening round to oval.
K11: 59	7	Metathoracic spiracle orientation: (0) dorsad; (1) posterolaterad; (2) anterolaterad.
K11: 60 (BU92: 11)	8	Metapleural gland: (0) absent; (1) present.
K11: 61	9	Ventral flap on metapleural gland opening: (0) absent; (1) bulla shaped and unarmed; (2) oblong and armed with a keellike projection.
K11: 62 (BU92: 12)	10	Metapleural longitudinal flange: (0) absent; (1) projecting laterad; (2) projecting ventrolaterad, concealing the metapleural gland opening.
K11: 64	11	Propodeal spiracle: (0) with bulla; (1) without bulla.
K11: 65	12	Propodeal spiracle atrial opening: (0) slit shaped; (1) round to oval.
K11: 67	13	Propodeal lobe: (0) absent; (1) present.
K11: 69	14	Metacoxal cavity: (0) open; (1) closed.
K11: 71	15	Stout setae on posterior apex of protibia: (0) absent; (1) one; (2) two.
K11: 72	16	Stout setae on posterior surface of probasitarsal noth: (0) absent; (1) single; (2) row parallel to strigil comb.
K11: 74	17	Calcar of strigil: (0) fully pectinated; (1) with a basal lamella; (2) fully lamellated.
K11: 76	18	Brush on posterior surface of strigil calcar: (0) absent; (1) present.
K11: 78	19	Squamiform brush on calcar anterior surface of strigil: (0) absent; (1) present.
K11: 80	20	Protarsus fourth tarsomere: (0) with lateral lobate projections; (1) conical; (2) cylindrical.
K11: 82	21	Claws in propretarsus: (0) simple; (1) with preapical tooth; (2) pectinate; (3) with basal spines.
K11: 83	22	Arolium in propretarsus: (0) vestigial to absent; (1) present.
K11: 84	23	Arolium in mesopretarsus and metapretarsus: (0) vestigial to absent; (1) present.
K11: 88	24	Anterior metatibial spur: (0) absent; (1) simple; (2) pectinate.
K11: 91	25	Mesobasitarsal sulcus: (0) absent; (1) present.
K11: 92	26	Metabasitarsal sulcus: (0) absent; (1) present.

Unused Characters		
BU92: 10	1	Worker. Promesonotal suture fused (1) or mobile (0).
BU92: 11	2	Worker and gyne. Metapleural gland present (1) or absent (0).
BU92: 12	3	Worker and gyne. Opening of the metapleural gland covered from above by a cuticular flange (1), or not (0).
BU92: 13	4	Worker, gyne and male. Metacoxal cavities open or sutured (0), or closed (1).
BU92: 14	5	Worker. 'Metatibial gland' present (1), or absent (0)
BU92: 15	6	Worker and gyne. Basitarsal sulcus on metatarsi present (1) or absent (0).
K11: 51	7	Stout, peglike setae on pronotal disc: (0) absent; (1) present.
K11: 54	8	Mesonotal lateral margins: (0) parallel to subparallel; (1) posteriorly tapering, dorsum triangular.
K11: 55	9	Median sulcus on mesepisterna: (0) absent; (1) present.
K11: 56	10	Mesothoracic spiracle: (0) concealed by a spiracular lobe; (1) exposed.
K11: 63	11	Propodeal lateral margins: (0) parallel to subparallel; (1) upper anterior part strongly constricted.
K11: 66	12	Propodeal spines: (0) absent; (1) present.
K11: 68	13	Propodeal foramen: (0) free and visible; (1) enclosed in tubular projection.
K11: 70	14	Protibial anterior sulcus: (0) absent; (1) present.
K11: 73	15	Basal projection on inner portion of probasitarsus: (0) absent; (1) rounded; (2) acute.
K11: 75	16	Lamella of strigil calcar: (0) entire; (1) with a notch.
K11: 77	17	Longitudinal sulcus on probasitarsal posterior surface: (0) absent; (1) present.
K11: 79	18	Setae on anterior surface of probasitarsal notch: (0) absent; (1) paddle shaped; (2) serrated.
K11: 81	19	Propretarsal manubrium: (0) flat and discoidal; (1) protruded distad.
K11: 85	20	Spinelike setae on midleg: (0) absent; (1) present on tibia and basitarsus; (2) present on basitarsus only.
K11: 86	21	Spinelike setae on metabasitarsus: (0) absent; (1) present.
K11: 87	22	Metacoxal dorsum: (0) unarmed; (1) armed with a tooth or spine.
K11: 89	23	Posterior metatibial spur: (0) absent; (1) simple; (2) pectinate.
K11: 90	24	Microtrichia on metatibial spur posterior face: (0) absent; (1) present, simple; (2) present, antlerlike.
K11: 93	25	Metabasitarsal ventral longitudinal groove: (0) absent; (1) present.
K11: 94	26	Basitarsal gland on metatarsus: (0) absent; (1) present.

Keller 2011 (K11)																												
67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	
13		14		15	16		17		18		19		20		21	22	23				24			25	26			
1	0	1	0	1	(0,1)	0	0	0	0	0	(0,1)	1	1	0	0	1	1	0	0	0	0	2	0	0	0	0	0	
1	0	0	0	1	2	0	(0,1)	0	0	0	1	1	1	0	1	0	0	0	0	0	0	(1,2)	0	0	0	0	0	
1	0	1	0	1	(1,2)	0	1	0	1	0	1	1	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	
(0,1)	0	1	0	0	(0,1)	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	
1	0	1	0	0	(0,2)	0	1	0	1	0	1	1	0	0	1	1	1	0	0	0	1	2	0	(0,1)	1	0	0	
1	0	0	0	(1,2)	(1,2)	0	1	0	0	0	1	1	0	0	1	1	1	0	0	0	(1,2)	2	0	1	1	0	0	
0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	2	0	0	0	0	0	
1	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	
1	0	1	0	0	0	0	0	0	0	0	0	(1,2)	1	0	0	1	1	0	0	0	0	2	0	0	0	0	0	
1	0	0	0	(0,1)	(1,2)	0	1	0	(0,1)	0	1	1	0	0	1	1	1	0	0	0	(1,2)	2	1	0	0	0	0	
1	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	2	0	0	0	0	0	
1	0	0	0	0	2	0	1	0	1	1	1	1	0	0	1	1	1	0	0	0	0	2	0	1	1	0	0	
1	0	1	0	0	0	0	0	0	0	0	1	1	2	0	0	1	1	0	0	0	0	2	0	0	0	0	0	
1	0	0	0	(0,1)	0	0	(0,1)	0	0	0	1	1	1	0	0	1	1	0	0	0	(0,1)	2	0	0	0	0	(0,1)	
0	0	1	0	0	0	0	0	0	0	0	?	?	1	0	0	1	1	0	0	0	1	2	0	0	0	0	0	
1	0	?	0	2	0	0	2	0	0	0	0	0	0	0	0	1	1	0	0	0	2	2	0	0	0	0	0	
?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
1	0	1	0	2	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	2	2	0	0	0	0	0	

e.

Study IV

Appendix 2: Muscular homology chart of Hymenoptera

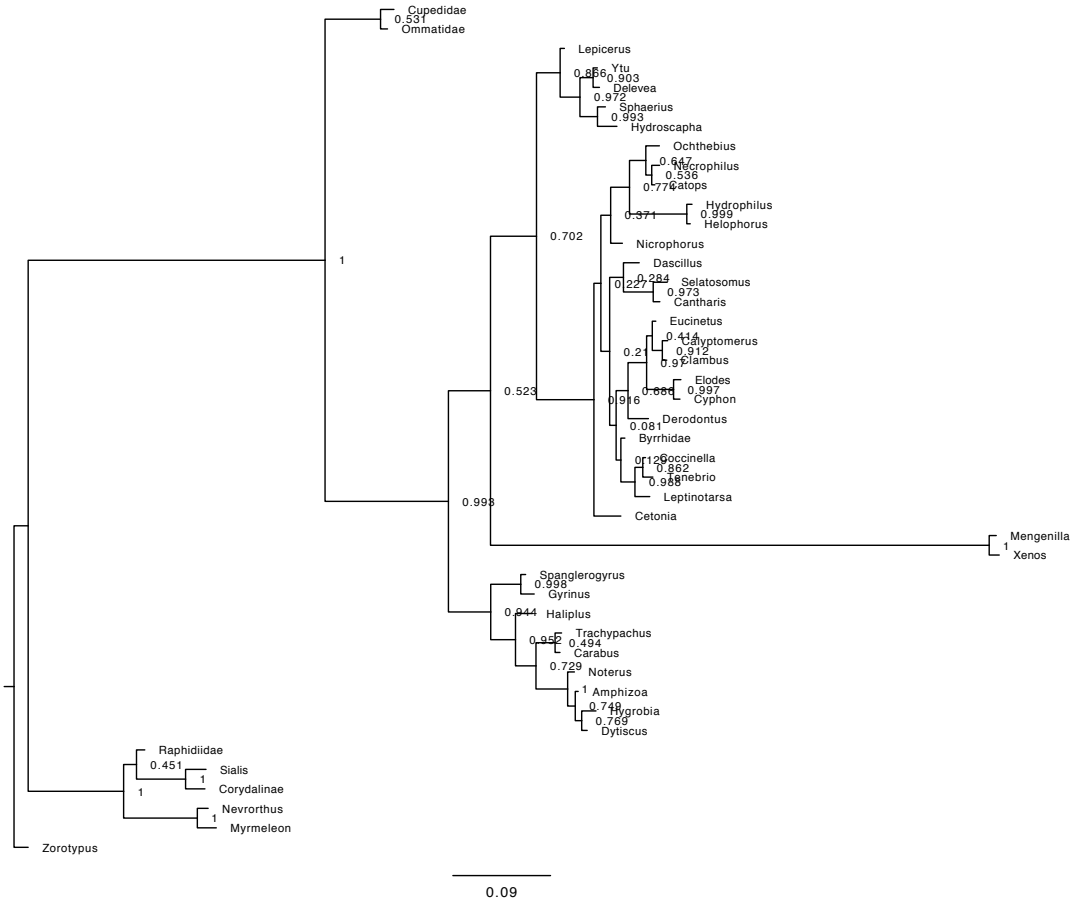
Reviewed and published versions are same.

Present with "+" or muscular name in green, absent with "-" in pink, uncertain with "?" or "/" in yellow. In Formicidae, the muscles only present in workers are labeled in dark green; muscles only occurring in alate castes labeled in dark blue.

Family	Xyelidae	Tenthredinidae	Braconidae	Ichneumonidae	Vespidae			Apidae	Formicidae			
Subfamily									Myrmeciinae		Formicinae	
Species	*Xyela julii*	*Eutomostethus f*	*Stenobracon dee*	*Philopsyche sau*	*Vespa ducali*	*Vespa vulgar*	*Vespula* sp.	*Apis mellifica*	*Myrmecia nigrocincta*	*Camponotus camelinus*	*Lasius flavus*	*Formica polyctena*
References	Vilhelmsen 2	Maki 1938	Alam 1951	Maki 1938	Maki 1938	Markl 1966	Duncan 1939	Snodgrass 1942	present study	Saini 1982	Lubbock 1881	Markl 1966
Idlm1	4	-	-	-	1	40+41	-	40+41	+	1 (absent in winged type	a	40&41
Idlm2	-	-	47	-	-	-	51	-	-	-	-	-
Idlm3	-	-	43?&44?	-	2	-	-	-	-	-	-	-
Idlm4	-	-	-	-	-	-	-	-	-	-	-	-
Idlm5	19	-	46	-	3	45	50	45	+	-	-	45
Idlm6	-	-	-	-	-	-	-	-	-	-	-	-
Idvm1	-	-	-	4?	-	-	-	-	-	-	-	-
Idvm2	1	-	-	4?&5?	-	-	-	-	-	-	-	-
Idvm3	1	-	-	5?	-	-	-	-	-	-	-	-
Idvm4	-	-	-	-	-	-	-	-	-	-	-	-
Idvm5	-	-	-	8	9	47	38	47	+	6	c	46&47
Idvm6	5	6	-	-	-	-	-	-	-	-	-	-
Idvm7	8?	-	51	7	8	46	-	46	-	-	-	-
Idvm8	8?	-	-	-	-	-	-	-	-	-	-	-
Idvm9	2	5	40	6	7	43	33	43	+	2	a1	43
Idvm10	20	-	-	-	-	-	-	-	-	-	-	-
Idvm11	-	-	-	-	-	-	-	-	-	-	-	-
Idvm12	-	-	-	-	-	-	-	-	-	-	-	-
Idvm13	-	-	-	-	-	-	-	-	-	-	-	-
Idvm14	-	-	-	-	-	-	-	-	-	-	-	-
Idvm15	-	-	-	-	-	-	-	-	-	-	-	-
Idvm16	11?	-	-	-	-	-	-	-	-	-	-	-
Idvm17	11?	-	-	-	-	-	-	-	-	-	-	-
Idvm18	-	12	57	13	16	55	48	55?	+	-	-	55
Idvm19	-	-	-	-	-	-	-	-	-	-	-	-
Itpm1	-	7	42	-	10	42/1	-	42b&c	+	-	b1	42/1
Itpm2	-	4	41	-	6	42/2	32	42a?	+	-	c1&c2	42/2
Itpm3	9	-	50	-	11	48	37	48	-	-	-	48
Itpm4	10	8	49	9	12	49	39-42	49	+	10	e	49
Itpm5	-	9	52&53	10	13	50	51	50	+	-	f	50
Itpm6	-	-	-	-	-	-	-	-	+	-	g	-
Ippm1	-	-	-	-	-	-	-	-	-	-	-	-
Ippm2	-	-	-	-	-	-	-	-	-	-	-	-
Ispm1	?	-	-	-	-	-	-	-	-	-	-	-
Ispm2	?	-	-	-	-	-	-	-	-	-	-	-
Ispm3	?	-	-	-	-	-	-	-	-	-	-	-
Ispm4	?	-	-	-	-	-	-	-	-	-	-	-
Ispm5	?	-	-	-	-	-	-	-	-	-	-	-
Ispm6	?	-	-	-	-	-	-	-	-	-	-	-
Ispm7	?	-	-	-	-	-	-	-	-	-	-	-
Ipcm1	?	10	-	11	14	mcr	49	pl 1-cx 1cX	-	-	d1	mcr
Ipcm2	7	-	-	-	-	-	-	-	-	-	-	-
Ipcm3	18?	-	59?	-	-	-	47	57?	-	-	-	-
Ipcm4	12	-	-	-	-	-	-	-	+	-	h	53
Ipcm5	-	16	55	16&17	19&20	53	44	53	-	-	-	-
Ipcm6	14	-	-	-	17	57	-	-	-	-	-	-
Ipcm7	-	-	-	-	-	-	-	-	-	-	-	-
Ipcm8	17?	17	61?	18	21	61	45?	61?	+	-	k	-
Ivlm1	6	2	54	2&3	5	51	36	51	+	-	d	51
Ivlm2	-	-	-	-	-	-	-	-	-	-	-	-
Ivlm3	3	1	45	1	4	44	34&35	44	+	3	b	44
Ivlm4	23	3	-	-	-	-	-	-	-	-	-	-
Ivlm5	-	-	-	-	-	-	-	-	-	-	-	-
Ivlm6	-	-	-	-	-	-	-	-	-	-	-	-
Ivlm7	21&22	22&23	48	22&23	24&25	52	53&54	52	+	18&19	m&n	52
Ivlm8	-	-	-	-	-	-	-	-	-	-	-	-
Ivlm9	24	-	-	-	-	-	-	-	-	-	-	-
Iscm1	13	11	56	12	15	54	43	54	+	-	i1?	54
Iscm2	14part	15	58?	15	18	56	-	56	-	-	i	56
Iscm3	-	-	-	-	-	-	-	-	+	-	-	-
Iscm4	15	14	58	-	-	-	-	-	+	-	-	-
Iscm5	16	13	60	14	-	58	52	58	-	-	-	-
Iscm6	17	-	61?	-	-	-	45?	61?	+	-	l	61
Iscm7	-	-	-	-	-	-	-	-	-	-	-	-
IIdlm1	?	18	62	19	22	/	56	71	-	20	β	-
IIdlm2	?	19&21	-	20	-	/	-	-	-	-	-	-
IIdlm3	2	20	70	21	23	/	72	70	-	-	-	-
IIdvm1	?	24	111	24	26	/	57	72	-	21	θ	-
IIdvm2	?	-	-	-	-	/	-	-	-	-	-	-
IIdvm3	?	-	-	-	-	/	-	-	-	-	-	-
IIdvm4	?	-	-	-	-	/	-	-	-	-	-	-
IIdvm5	?	-	-	-	-	/	-	-	+	-	-	-
IIdvm6	?	36	72	34	-	/	-	82	-	23	-	-
IIdvm7	?	-	-	-	-	/	-	-	-	-	o	-
IIdvm8	?	25&26	63	25	27	/	58	78	-	-	-	-
IIdvm9	?	-	-	-	-	/	-	-	-	-	-	-
IItpm1	?	27	-	-	-	/	-	-	-	-	-	-
IItpm2	?	28	-	-	-	/	-	74	-	-	-	-
IItpm3	?	-	-	-	-	/	-	-	-	-	-	-
IItpm4	?	-	-	-	-	/	-	-	-	-	-	-
IItpm5	?	29	68?	26	28	/	62	75	-	-	-	-
IItpm6	?	-	-	-	-	/	-	-	-	25	-	-
IItpm7	?	30	66&67	27&28	29&30	/	60&61	76	-	24a	-	-
IItpm8	?	-	-	-	-	/	-	-	-	-	-	-
IItpm9	?	31	-	29	-	/	-	-	-	24b	-	-
IItpm10	?	-	-	-	-	/	-	-	-	-	-	-
IItpm11	?	-	-	-	31	/	64	-	-	-	-	-
IItpm12	?	-	-	-	-	/	-	-	-	-	-	-
IIppm1	?	-	-	-	-	/	-	-	-	-	-	-
IIppm2	?	-	-	-	-	/	-	-	-	-	-	73
IIspm1	?	32	64	30	32	/	59	77	-	-	-	-
IIspm2	?	33&34	69	32	33&34	/	70	79	-	-	-	-
IIspm3	?	-	-	-	-	/	-	-	-	-	-	-
IIspm4	?	-	-	-	-	/	-	-	-	-	-	-
IIspm5	?	-	-	-	-	/	-	-	-	-	-	-
IIspm6	29	52	-	45	-	/	-	-	-	-	-	-
IIspm7	?	-	-	-	-	/	-	-	-	-	-	-
IIspm8	?	-	-	-	-	/	-	-	-	-	-	-
IIpcm1	?	-	-	-	-	/	-	-	-	-	-	-
IIpcm2	?	-	-	31	-	/	-	-	-	22	-	-

IIpcm3	?	38	73	-	36	/	-	80	-	-	-	-
IIpcm4	?	-	-	-	-	/	-	-	-	-	-	-
IIpcm5	?	-	-	-	-	/	-	-	-	-	-	-
IIpcm6	?	-	-	-	-	/	-	-	-	-	-	-
IIvlm1	?	-	-	-	-	/	-	-	-	-	-	-
IIvlm2	?	-	-	-	-	/	-	-	-	-	-	-
IIvlm3	27	44	71	40	40	/	73	-	-	-	-	79?
IIvlm4	?	-	-	-	-	/	-	-	-	-	-	-
IIvlm5	28	-	-	-	-	/	-	-	-	-	-	-
IIvlm6	?	-	-	-	-	/	-	-	-	-	-	-
IIvlm7	?	-	-	-	-	/	-	-	+	35	v	118
IIscm1	?	35	74	33	35	/	65	81	+	28	p&s	81&82
IIscm2	?	37	75	35	36	/	68	83?	-	-	q	83
IIscm3	?	-	75	-	-	/	68	83?	+	29&31	r	83
IIscm4	?	-	-	-	-	/	-	-	+	30	t	80
IIscm5	?	-	-	-	-	/	-	-	-	-	-	-
IIscm6	?	39	76	37	37	/	67	86	-	-	o	86
IIscm7	?	-	-	-	-	/	-	-	-	-	-	-
IIIdlm1	5	41	-	39	39	/	75	96	-	-	-	-
IIIdlm2	6	42	-	-	-	/	-	-	-	-	-	-
IIIdlm3	?	43	-	-	-	/	-	-	-	-	-	-
IIIdvm1	9	45	-	-	-	/	-	97?&98?&99?	-	-	θ	-
IIIdvm2	17	-	-	-	-	/	-	-	-	-	-	-
IIIdvm3	-	-	-	-	-	/	-	-	-	-	-	-
IIIdvm4	18	-	-	-	-	/	-	-	+	40	y?	103
IIIdvm5	19	-	-	-	-	/	-	-	+	-	y?	105
IIIdvm6	22	57	81	51	46	/	80	105	-	37	-	-
IIIdvm7	20?	-	-	-	-	/	-	-	-	-	x	-
IIIdvm8	7&8	45a	-	-	-	/	-	-	-	-	-	-
IIItpm1	3	46	-	-	-	/	-	-	-	-	-	-
IIItpm2	4	47	-	41	-	/	-	-	-	-	-	-
IIItpm3	11	-	-	-	-	/	-	-	-	-	-	-
IIItpm4	-	-	-	-	-	/	-	-	-	-	-	-
IIItpm5	10	48	84?&85?&86?	42	41	/	79	-	-	-	-	-
IIItpm6	-	-	-	-	-	/	-	-	-	39	-	-
IIItpm7	12a	49	88	43	42	/	-	100	-	38a	-	-
IIItpm8	-	-	-	-	-	/	-	-	-	-	-	-
IIItpm9	12b	50	89	-	-	/	77	-	-	38b	-	-
IIItpm10	15	-	-	-	-	/	-	-	-	-	-	-
IIItpm11	-	51	82	44	43	/	78	102	-	-	-	-
IIItpm12	-	-	-	-	-	/	-	-	-	-	-	-
IIIppm1	14?	-	-	-	-	/	-	-	-	-	-	-
IIIppm2	-	-	-	-	-	/	-	-	-	-	-	-
IIIspm1	13	53	87	46&47	44	/	76	101	-	-	-	-
IIIspm2	23	54&55	-	48	-	/	-	-	-	-	-	-
IIIspm3	-	-	-	-	-	/	-	-	-	-	-	-
IIIspm4	-	-	-	-	-	/	-	-	-	-	-	-
IIIspm5	-	-	-	-	-	/	-	-	-	-	-	-
IIIpcm1	-	-	-	-	-	/	-	-	-	-	-	-
IIIpcm2	-	59	-	-	-	/	-	-	-	36	-	-
IIIpcm3	21?	-	77b	53	48	/	84	103?	-	-	-	-
IIIpcm4	21?	-	-	54	-	/	-	-	-	-	-	-
IIIpcm5	26	-	-	-	-	/	-	-	-	-	-	-
IIIpcm6	-	-	-	-	-	/	-	-	-	-	-	-
IIIpcm7	-	-	-	-	-	/	-	-	-	-	-	-
IIIvlm1	-	-	-	-	-	/	-	-	-	-	-	-
IIIvlm2	/	/	/	/	/	/	/	/	+	33	u1	119
IIIvlm3	35	-	-	-	-	/	-	-	-	-	-	-
IIIscm1	-	56	78	45	49	/	81	104	+	41	z	104&105
IIIscm2	25	58	79	52	47	/	82	106?	-	-	x1	106
IIIscm3	30	-	-	-	-	/	-	106?	-	43	z1	106
IIIscm4	-	-	-	-	-	/	-	-	+	42	y1	103
IIIscm5	24	-	80?	-	-	/	-	-	-	-	-	-
IIIscm6	-	60	83	55	49	/	83	109	-	-	x	109
IA1	/	/	/	/	/	/	/	/	+	34	u	120
IA2	/	/	/	/	/	/	/	/	+	32	w	121

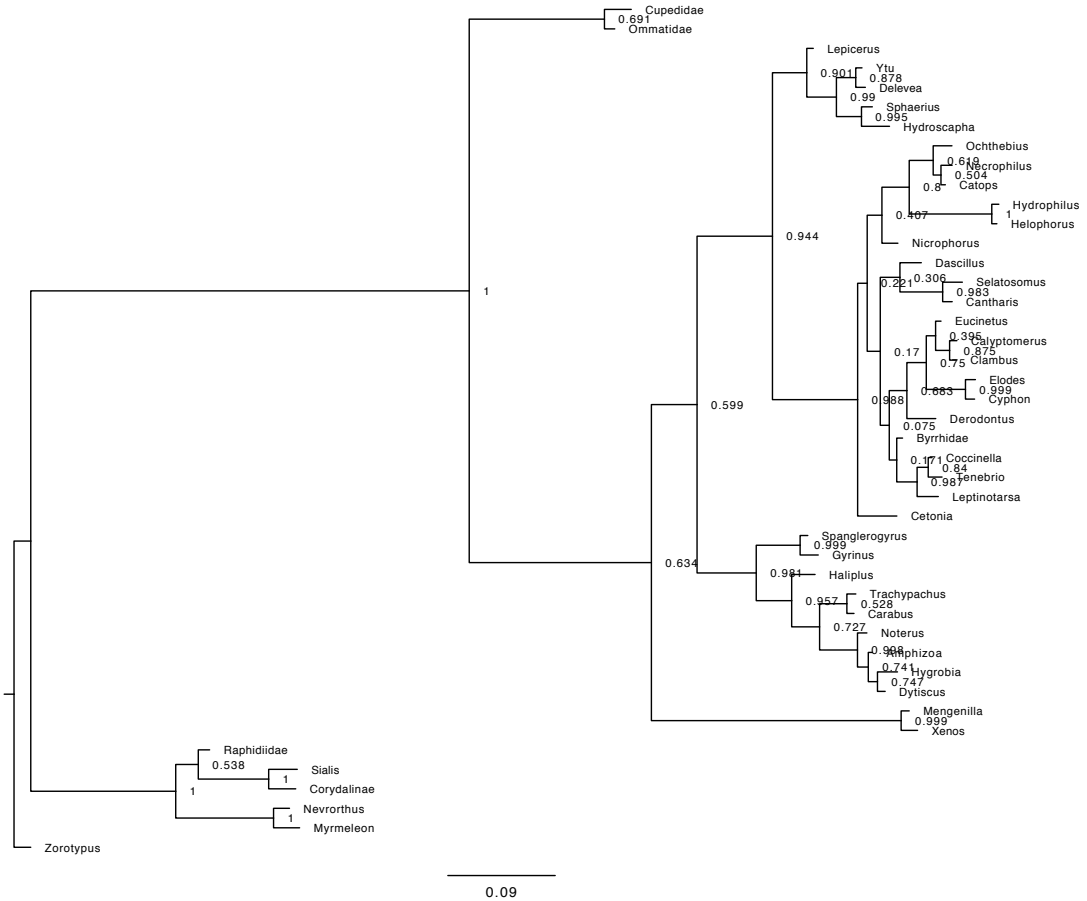
Study V



Appendix 3: Fig. A1. Bayesian tree 190 characters.

Reviewed and published versions are same.

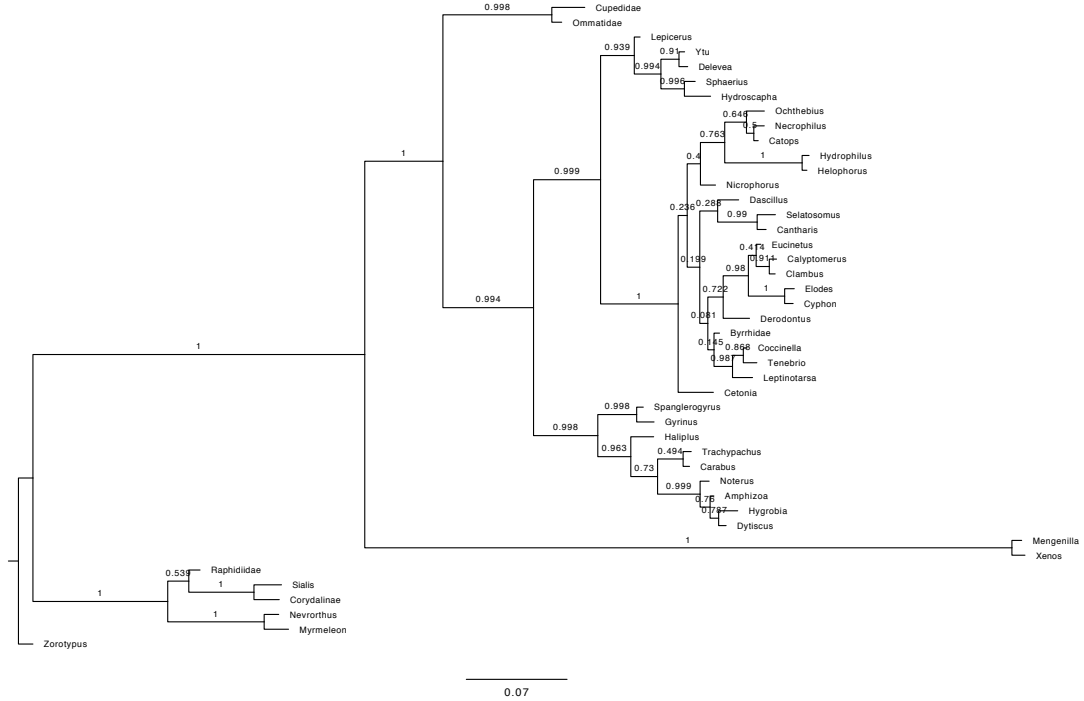
Posterior probabilities above branches.



Appendix 4: Fig. A2. Bayesian tree. 156 characters.

Reviewed and published versions are same.

With 34 characters excluded, all of them presumptive autapomorphies of Strepsiptera (chars. 1, 13, 16, 29, 33, 35, 37, 40, 42, 50, 53, 56, 59, 63, 67–69, 75, 77, 83, 84, 107, 145, 146, 152, 165, 175, 176, 184–188, 190). Posterior probabilities above branches.



Appendix 5: Fig. A3. Bayesian tree. 190 characters.

Reviewed and published versions are same.

Monophyletic Coleoptera enforced.

Study V

Appendix 6: Homology of thoracic muscles

Reviewed and published versions are same.

	present study = (based on Beutel and Haas, 2000)	Baehr, 1975	Larsén, 1966	Koeth et al., 2012	Friedrich and Beutel, 2008
prothorax	M. 1	2	M1	m2	Idlm2
	M. 2	1	M2	m1	Idlm1
	M. 3	3	M3	-	Idlm3
	K5	-	-	m5	Idlm4?
	M. 4	-	-	-	Idlm1?
	M. 5	-	-	-	Idvm7?
	M. 6	4	M4	m3	Idlm5
	M. 7	-	-	-	Itpm1
	M. 8	-	-	-	Idvm1?
	M. 9	5	M5	m13	Ivlm1
	M. 10	8	M6	-	Ivlm3
	M. 11	10	-	-	Idvm9
	M. 12	6	M7	-	Idvm6
	M. 13	-	M8	m6	Idvm8
	M. 14	7	M9	-	Idvm5
	M. 15	9?	M10	m4	Idvm2, 3
	M. 16	13	M11	m7	Idvm10
	M. 17	11	M12	-	Itpm3?
	M. 18	12	M13	-	Itpm6
	M. 19	-	-	-	Itpm12?
	M. 20	35	-	-	Ispm2
	M. 21	-	-	-	Ivlm4
	M. 22	14	M14	m8	Idvm13
	M. 23	15	M15	m9	Idvm16, 17
	M. 24	16	M16	-	Ipcm4
	K11	-	-	m11	Ipcm6
	M. 25	17?	M17	-	Idvm18
	M. 26	-	-	-	Ipcm1
	M. 27	18	M18	m15	Iscm1
	M. 28	-	-	-	Iscm5
	M. 29	-	-	-	Ipcm7
	M. 30	19	M19	m16	Iscm2
	M. 31	20	M20	-	Ipcm8
mesothorax	M. 39	24	M28	m17	IIdlm1
	M. 40	24	M29	m18	IIdlm2
	M. 41	-	-	-	IIdlm3
	M. 42	25	M30	m14	Ivlm7
	M. 43	26	M31	-	Ivlm9
	M. 44	27	-	m19	IIdvm1
	M. 45	32	M32	m21	IIdvm8
	M. 46	28	-	-	IItpm6
	M. 47	29	M33	-	IItpm2
	M. 48	-	-	-	IItpm3
	M. 49	-	-	-	IItpm4
	M. 50	33	-	m22	IIspm1
	M. 52	-	M35, M34?	-	IItpm10
	M. 53	31	M36b	-	IItpm7
	M. 54	30	M36a	-	IItpm9
	M. 55	34	M37	-	IIspm2
	M. 56	-	-	-	IIppm1
	M. 57	-	-	-	?
	M. 58	-	M38	-	Ispm6
	M. 59	36	M39	-	IIdvm2
	M. 60	37	M40	-	IIdvm5, 4?
	M. 61	38	M41	m23	IIpcm4
	M. 62	39	M42	-	IIpcm3
	M. 63	-	-	-	IIscm1?
	M. 64	40	M43	m20	IIdvm6
	M. 65	41	M44	m26	IIscm1
	M. 66	42	M45	-	IIscm4
	M. 67	43	M46	m27	IIscm2
	M. 68	-	-	-	IIpcm2
	M. 69	44	M47	-	IIdvm7
	M. 70	45	M48	-	IIpcm6
	M. 71	46	M50	-	IIpcm5
	M. 72	47	M52	-	IIscm6
metathorax	M. 79	50	M60	m28	IIIdlm1
	M. 80	51	M61	m29	IIIdlm2
	M. 81	-	-	-	IIIdlm3
	M. 82	52	M62	m25	IIvlm3
	M. 83	53	M63	-	IIvlm5
	M. 84	55	M64	m30	IIIdvm1
	M. 85	62	M65	m33	IIIdvm8
	M. 86	63	M66	m33	IIIdvm8
	M. 87	67	-	-	IIspm3
	M. 88	68	-	-	IIspm6
	M. 89	56	-	-	IIItpm1
	M. 90	58	M67	m34	IIItpm2
	M. 91	-	-	m35	IIItpm5
	M. 92	59	M68	-	IIItpm6
	M. 93	57	M69	-	IIItpm3

M. 94	66	M70	-	IIItpm10
M. 95	60	M71a	m37	IIItpm9
M. 96	61	M71b	m36	IIItpm7
M. 97	64	M72	m38	IIIppm1
K39	-	-	m39	IIIppm2
M. 98	65	M73	-	IIIspm1
M. 99	-	-	m40	IIIspm2
M. 100	69	M74	-	IIIdvm2
M. 101	70	M75	m31	IIIdvm4
M. 102	41	M76	-	IIIdvm5
M. 103	72	M77	-	IIIpcm4
M. 104	73	M78	m41	IIIpcm3
M. 105	74	M79	m32	IIIdvm6
M. 106	-	M80	-	IIscm7?
M. 107	75	M81	-	IIIscm1
M. 108	76	M82	-	IIIscm4
M. 109	77	M83	-	IIIscm2
M. 110	-	-	-	IIIpcm2
M. 111	78	M84	-	IIIdvm7
M. 112	79	-	-	IIIpcm5
M. 113	80	M85	-	IIIscm6
fam	54	n.a.	m42/43	IIIvlm2

9. Publications

Liu, S.-P. & Yang, D. (2012) Two new species of *Hoplosathe* Lyneborg & Zaitzev, 1980 (Diptera: Therevidae: Therevinae) in China. *Entomotaxonomia,* 34(2): 313–319.

Liu, S.-P., Li, Y. & Yang, D. (2012) One new species of *Bugulaverpa* Gaimari & Irwin, 2000 (Diptera: Therevidae: Therevinae: Cyclotelini) from China. *Entomotaxonomia,* 34(3): 545–549.

Liu, S.-P. & Yang, D. (2012) Revision of the Chinese species of *Dialineura* Rondani, 1856 (Diptera, Therevidae, Therevinae). *Zookeys*, 235: 1–22.

Liu, S.-P., Gaimari, S.D. & Yang, D. (2012) Species of *Ammothereva* Lyneborg, 1984 (Diptera: Therevidae: Therevinae: Cyclotelini) from China. *Zootaxa*, 3566: 1–13.

Liu, S.-P., Wipfler, B., Niitsu, S. & Beutel, R.G. (2017) The thoracic anatomy of the male and female winter moth *Nyssiodes lefuarius* (Lepidoptera: Geometridae) and evolutionary changes in the thorax of moths and butterflies. *Organisms Diversity & Evolution*, 17(3): 565–594.

Liu, S.-P., Wipfler, B. & Beutel, R.G. (2018) The unique locomotor apparatus of whirligig beetles of the tribe Orectochilini (Gyrinidae, Coleoptera). *Journal of Zoological Systematics and Evolutionary Research*, 56(2): 196–208.

Liu, S.-P., Friedrich, F., Petersen, D.S., Büsse, S., Gorb, S.N. & Beutel, R.G. (2018) The thoracic anatomy of the swift lousefly *Crataerina pallida* (Diptera) – functional implications and character evolution in Hippoboscidea. *Zoological Journal of the Linnean Society*, (https://doi.org/10.1093/zoolinnean/zly032).

Beutel, R.G., Pohl, H.W., Yan, E.V., Anton, E., **Liu, S.-P.**, Slipinski, A., McKenna, D. & Friedrich, F. (2018) The phylogeny of Coleopterida (Hexapoda) – morphological characters and molecular phylogenies. *Systematic Entomology*, (DOI: 10.1111/syen.12316).

Liu, S.-P., Richter, A. Stößel, A. & Beutel, R.G. (2019) The mesosomal anatomy of *Myrmecia nigrocincta* workers and evolutionary transformations in Formicidae (Hymenoptera). *Arthropod Systematics & Phylogeny*, (accepted).

10. Acknowledgements

I firstly appreciate Prof. Dr. Rolf G. Beutel (Intitut für Zoologie und Evolutionsforschung, Friedrich-Schiller-Universität Jena), who recruited me as a PhD candidate in his laboratory. He provided me this brilliant project under the BIG4 programme with many chances to attend the academic communications around Europe. Also, he always instructed me how to organize a research and write a scientific paper in morphological field, which I had never practiced before arriving at Germany. Meanwhile, he is extremely knowledgeable not only in entomology, but also in literature, arts and languages, which always made me surprise and admire very much.

Secondly, I appreciate Prof. Dr. Martin S. Fischer (Intitut für Zoologie und Evolutionsforschung, Friedrich-Schiller-Universität Jena), the director of our institute. He attended my presentation and discussed many interesting questions with me, which trigger me a deeper thinking of my project. His students always gave me many helps to use the computer of their laboratory, which was a very happy time.

I need to thank Dr. Benjamin Wipfler (Intitut für Zoologie und Evolutionsforschung, Friedrich-Schiller-Universität Jena), who is very good at the using of modern morphological techniques. He kindly introduced me how to use a series of morphological softwares such as Amira, VGStudio when I began my project as a rookie. I need to thank PD Dr. Hans Pohl who patiently taught me how to use the complicated scanning electron microscopy (SEM). I need to thank Dr. Frank Friedrich (Institut für Zoologie, Universität Hamburg). He kindly gave me a very big help in morphological phylogeny. He also invited me to his cozy laboratory, where I made many young friends.

I felt very grateful that when I firstly arrived at Jena without any knowledge about German language, my laboratory mates Dr. Katharina Schneeberg (Pfalzmuseum für Naturkunde), Dr. Margarita I. Yavorskaya (Intitut für Zoologie und Evolutionsforschung, Friedrich-Schiller-Universität Jena) helped to apply the bank and insurance accounts. The cooperations with the Japanese winter moth expert Dr. Shuhei Niitsu (Department of Biology, Tokyo Metropolitan University), the biomechanical team including Dipl. Bio. Dennis S. Petersen, Dr. Sebastian Büsse and their supervisor Prof. Dr. Stanislav N. Gorb (Zoologisches Institut, Christian-Albrechts-Universität zu Kiel) and the ant expert Mr. Adrian Richter (Intitut für Zoologie und Evolutionsforschung, Friedrich-Schiller-Universität Jena) were excellent. I also need to bring my sincere appreciation to all of them.

As an exchange student in Chinese Academy of Sciences, Prof. Dr. Ming Bai (Institute of Zoology, Chinese Academy of Sciences) and his research assistant M.Sc. Hai-Dong Yang

taught how to use the softwares about geometric morphometrics and analyze the research result. The laboratory technician M.Sc. Cai-Xia Gao helped me to use the micro-CT. Of course I need to mention Prof. Dr. Chao-Dong Zhu (Institute of Zoology, Chinese Academy of Sciences) kindly invited me to attend the "Insect phylogenetic and systematics training course".

Finally I send my sincere regards to all the members of BIG4, including the project leader Prof. Dr. Alexey Solodovnikov (Zoological Museum, Natural History Museum of Denmark), the supervisors, the students and the administrative staffs, who always give me the biggest support. Especially I need to thank M. Sc. Trevor O. Burt (Zoologische Forschungsmuseum Alexander König) and Dipl. Bio. Anne-Sarah Ganske (Naturhistorisches Museum Wien) to help me in English expression and German translation. This material reflects only the author's view and the Research Executive Agency is not responsible for any use that may be made of the information it contains. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No. 642241. (http://big4-project.eu)

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